

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number
WO 02/29050 A2

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/72

(21) International Application Number: PCT/EP01/11442

(22) International Filing Date: 4 October 2001 (04.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/238,045 6 October 2000 (06.10.2000) US
60/315,958 31 August 2001 (31.08.2001) US

(71) Applicant (for all designated States except US): **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **LIOU, Jiing-Ren**
[CN/US]; 10 Winslow Road, Belmont, MA 02478 (US).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

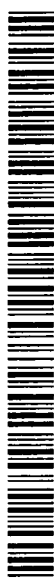
(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/29050 A2

(54) Title: RECEPTOR-LIKE GPCR SECRETIN RECEPTOR-LIKE GPCR

(57) Abstract: Reagents which regulate human secretin receptor-like GPCR and reagents which bind to human secretin-like GPCR gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, urinary incontinence, human prostate hyperplasia, obesity and fatty acids related to obesity, cancer, diabetes, osteoporosis, anxiety, depression, Parkinson's disease, Huntington's disease, Alzheimer's disease, and other neurodegenerative diseases.

REGULATION OF HUMAN SECRETIN RECEPTOR-LIKE GPCR

This application claims the benefit of and incorporates by reference co-pending provisional application Serial No. 60/238,045 filed October 6, 2000.

5

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of regulation of G protein-coupled receptors.

10 BACKGROUND OF THE INVENTION

G Protein-Coupled Receptors

Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature* 351, 353-354, 1991). The family of G protein-coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as calcitonin, adrenergic hormones, endothelin, cAMP, adenosine, acetylcholine, serotonin, dopamine, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G proteins themselves, effector proteins such as phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

25 The GPCR protein superfamily now contains over 250 types of paralogues, receptors that represent variants generated by gene duplications (or other processes), as opposed to orthologues, the same receptor from different species. The superfamily can be broken down into five families: Family I, receptors typified by rhodopsin and the β 2- adrenergic receptor and currently represented by over 200 unique members (reviewed by Dohlman *et al.*, *Ann. Rev. Biochem.* 60, 653-88, 1991, and references therein); Family II, the recently characterized parathyroid

30

hormone/calcitonin/secretin receptor family (Juppner *et al.*, *Science* 254, 1024-26, 1991; Lin *et al.*, *Science* 254, 1022-24, 1991); Family III, the metabotropic glutamate receptor family in mammals (Nakanishi, *Science* 258, 597-603, 1992); Family IV, the cAMP receptor family, important in the chemotaxis and development of *D. discoideum* (Klein *et al.*, *Science* 241, 1467-72, 1988; and Family V, the fugal mating pheromone receptors such as STE2 (reviewed by Kurjan, *Ann. Rev. Biochem.* 61, 1097-1129, 1992).

GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitoylation or farnesylation) of cysteine residues can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the β -adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydrophilic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and having a ligand binding site, such as the TM7 aspartate residue, TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (*see Johnson et al., Endoc. Rev. 10, 317-331, 1989*). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the effect of hormone binding is the activation inside the cell of the enzyme, adenylate cyclase. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G protein connects the hormone receptor to adenylate cyclase. G protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G protein itself, returns the G protein to its basal, inactive form. Thus, the G protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

Over the past 15 years, nearly 350 therapeutic agents targeting GPCRs receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is an ongoing need for identification and characterization of further GPCRs which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

Secretin

Secretin, a hormone from the duodenum, is a heptacosipeptide of the formula:

H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-
Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH₂ (Fig. 4) . Secretin stimulates the
pancreatic secretion of water and bicarbonate. U.S. Patent No. 4,098,779. In the
stomach, secretin stimulates pepsin secretion, stimulates the pyloric sphincter,
inhibits gastrin-stimulated acid secretion, inhibits food-stimulated gastrin release, and
inhibits motility. Rayford et al, New England Journal of Medicine, May 13, 1976
(1093-2000); U.S. Patent No. 4,086,220; U.S. Patent No. 4,711,847. For these
reasons, secretin promises to be a good medicament for gastrointestinal disorders,
such as, for example, for lesions in the gastrointestinal tract. Secretin also stimulates
cyclic AMP formation in the brain. Freneau *et al.*, *J. Neurochem.* 46, 1947-55,
1986.

Secretin exerts its effects through a type II GPCR. Shetzline *et al.*, *J. Biol. Chem.*
273, 6756-62, 1998; Chow, *Biochem. Biophys. Res. Commun.* 212, 204-11, 1995;
Ishihara *et al.*, *EMBO J.* 10, 1635-41, 1991; Jiang & Ulrich, *Biochem. Biophys. Res.*
Commun. 207, 883-90, 1995; Patel *et al.*, *Mol. Pharmacol.* 47, 467-73; Vilardaga *et*
al., *Mol. Pharmacol.* 45, 1022-28, 1994. Secretin receptor gene expression has been
shown to be upregulated in rat cholangiocytes after bile duct ligation. Alpini *et al.*,
Am. J. Physiol. 266, G922-28, 1994.

Because of the diverse biological effects of secretin and its receptor, there is a need in
the art to identify additional members of the secretin receptor family whose activity
can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a
human secretin receptor-like GPCR. This and other objects of the invention are
provided by one or more of the embodiments described below.

One embodiment of the invention is a secretin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

- 5 amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

- 10 amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 10; and

the amino acid sequence shown in SEQ ID NO:10.

- 15 Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a secretin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

- 20 amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

- 25 amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 10; and

the amino acid sequence shown in SEQ ID NO:10.

- 30 Binding between the test compound and the secretin receptor-like GPCR polypeptide is detected. A test compound which binds to the secretin receptor-like GPCR poly-

peptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the secretin receptor-like GPCR.

5 Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a secretin receptor-like GPCR polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

10

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

15

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

the nucleotide sequence shown in SEQ ID NO: 9.

20

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the secretin receptor-like GPCR through interacting with the secretin receptor-like GPCR mRNA.

25

Another embodiment of the invention is a method of screening for agents which
secretin receptor-like GPCR polypeptide comprising an amino acid sequence selected
30 from the group consisting of:

amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

5

amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 10; and

the amino acid sequence shown in SEQ ID NO:10.

10

A secretin receptor-like GPCR activity of the polypeptide is detected. A test compound which increases secretin receptor-like GPCR activity of the polypeptide relative to secretin receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases secretin receptor-like GPCR activity of the polypeptide relative to secretin receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

15

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a secretin receptor-like GPCR product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

20

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

25

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

30

the nucleotide sequence shown in SEQ ID NO: 9.

Binding of the test compound to the secretin receptor-like GPCR product is detected.

5 A test compound which binds to the secretin receptor-like GPCR product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a
10 polynucleotide encoding a secretin receptor-like GPCR polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

15 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

20 nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 9; and

the nucleotide sequence shown in SEQ ID NO: 9.

Secretin receptor-like GPCR activity in the cell is thereby decreased.

25 The invention thus provides a human secretin receptor-like GPCR which can be used to identify test compounds which may act as agonists or antagonists at the receptor

30 using specific antibodies which can block the receptor and effectively prevent ligand binding.

BRIEF DESCRIPTION OF THE DRAWING

- Fig. 1 shows the DNA-sequence encoding a secretin receptor-like GPCR polypeptide (SEQ ID NO:1).
- 5 Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).
- Fig. 3 shows the amino acid sequence of the protein identified by trembl Accession No. AB109120 (SEQ ID NO:3).
- Fig. 4 shows the amino acid sequence of a secretin receptor-like GPCR Polypeptide.
- 10 Fig. 5 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:4).
- Fig. 6 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:5).
- 15 Fig. 7 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:6).
- Fig. 8 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:7).
- Fig. 9 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:8).
- 20 Fig. 10 shows the DNA-sequence encoding a secretin receptor-like GPCR Polypeptide (SEQ ID NO:9).
- Fig. 11 shows the amino acid sequence deduced from the DNA-sequence of Fig.10 (SEQ ID NO:10).
- 25 Fig. 12 shows the BLASTP alignment of human secretin receptor-like GPCR (SEQ ID NO:2) with SEQ ID NO:3.
- Fig. 13 shows the HMMPFAM - alignment of SEQ ID NO:2 against pfam|hmm|7tm_2 7 transmembrane receptor (Secretin family) .
- Fig. 14 shows the HMMPFAM - alignment of SEQ ID NO:2 against pfam|hmm|GPS Latrophilin/CL-1-like GPS domain.
- 30 Fig. 15 shows the BLOCKS search results.

Fig. 16 shows the TBLASTN-alignment of 193 against bayer_dna|10895|10895

Figs. 17A, B and C show the expression profiling of secretin receptor-like GPCR polypeptide mRNA in different tissues.

5 Fig. 18 shows the gene expression of secretin receptor-like GPCR polypeptide mRNA in different tissues which are relevant for diabetes. The expression was determined in a RT-PCR with 35 cycles using gene specific primers. mRNA from the following tissues was analyzed: lane 1-adipose sub; lane 2-adipose mes; lane 3-islets; lane 4-hypo-
10 thalamus; lane 5-skeletal muscle; lane 6-liver; lane 7-genomic DNA; lane 8-NAC and lane 9-NTC. A positive signal is visible in the lanes: 2, 3, 4, 5 and 7.

DETAILED DESCRIPTION OF THE INVENTION

15

The invention relates to an isolated polynucleotide encoding a secretin receptor-like GPCR polypeptide and being selected from the group consisting of:

- 20 a) a polynucleotide encoding a secretin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 40% identical to
the amino acid sequence shown in SEQ ID NO: 2;
the amino acid sequence shown in SEQ ID NO: 2;
amino acid sequences which are at least about 40% identical to
25 the amino acid sequence shown in SEQ ID NO: 10; and
the amino acid sequence shown in SEQ ID NO:10.
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1 or 9;
polynucleotide specified in (a) and (b).

- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a novel secretin-like GPCR, particularly a human secretin-like GPCR, is a discovery of the present invention. Human secretin-like GPCR comprises the amino acid sequence shown in SEQ ID NOS:2 and 10. Human secretin-like GPCR is 35% identical over 617 amino acids to the rat protein identified with trembl Accession No. AB109120 and annotated as a "seven transmembrane receptor" (Fig. 12). Domains of human secretin-like GPCR are shown in Figs. 13-15.

A coding sequence for SEQ ID NOS:2 and 10 is shown in SEQ ID NOS:1 AND 10 and is located on chromosome 6. Genomic sequences are found in clones identified with GenBank Accession Nos. AL35621, AL358178, and AL161776. Related ESTs (SEQ ID NOS:4 to 8) are expressed in uterus and breast.

Human secretin-like GPCR also may be useful for the same purposes as previously identified GPCRs. Thus, human secretin-like GPCR may be used in therapeutic methods to treat disorders such as anxiety, depression, hypertension, osteoporosis, diabetes, cancer, migraine, compulsive disorders, schizophrenia, autism, neuro-degenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, urinary incontinence, benign prostate hyperplasia, obesity, and cancer chemotherapy-induced vomiting. Human secretin-like GPCR also can be used to screen for human secretin-like GPCR agonists and antagonists.

Polypeptides

Human secretin-like GPCR polypeptides according to the invention comprise at least 6, 8, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 600, 625, 650, 675, or 700 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NOS:2 and 10 or a biologically active variant thereof, as defined below. A human secretin-like GPCR polypeptide of the invention therefore can be a portion of a secretin receptor-like GPCR protein, a full-length secretin receptor-like GPCR protein, or a fusion protein comprising all or a portion of a secretin receptor-like GPCR protein. A coding sequence for SEQ ID NOS:2 and 10 is shown in SEQ ID NOS:1 and 10.

Biologically Active Variants

Secretin-like GPCR polypeptide variants which are biologically active, e.g., retain the ability to bind a ligand to produce a biological effect, such as cyclic AMP formation, mobilization of intracellular calcium, or phosphoinositide metabolism, also are secretin receptor-like GPCR polypeptides. Preferably, naturally or non-naturally occurring secretin receptor-like GPCR polypeptide variants have amino acid sequences which are at least about 40, 45, 50, 55, 60, 65, 70, preferably 75, 80, 85, 90, 95, 97, 98, or 99% identical to the amino acid sequence shown in SEQ ID NOS:2 and 10 or a fragment thereof. Percent identity between a putative secretin receptor-like GPCR polypeptide variant and an amino acid sequence of SEQ ID NOS:2 and 10 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions. Substitutions may be conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative

replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a secretin receptor-like GPCR polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active secretin receptor-like GPCR polypeptide can readily be determined by assaying for binding to a ligand or by conducting a functional assay, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against secretin receptor-like GPCR polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of secretin receptor-like GPCR polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A secretin receptor-like GPCR polypeptide fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 6, 8, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 600, 625, 650, 675, or 700 contiguous amino acids of SEQ ID NOS:2 and 10 or of a biologically active variant, such as those described above. The first polypeptide segment also can comprise full-length secretin receptor-like GPCR protein.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, 5 horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 10 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the secretin receptor-like GPCR polypeptide-encoding sequence and the heterologous protein sequence, so that the secretin receptor-like GPCR polypeptide can be cleaved and purified away from the heterologous moiety.

15 A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct 20 which comprises coding sequences selected from SEQ ID NOS:1 AND 10 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain 25 View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human secretin-like GPCR polypeptide can be obtained using secretin receptor-like GPCR polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of secretin receptor-like GPCR polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

10

A secretin receptor-like GPCR polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for secretin receptor-like GPCR polypeptide. A coding sequence for human secretin-like GPCR is shown in SEQ ID NOS:1 and 9.

15

Degenerate nucleotide sequences encoding human secretin-like GPCR polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NOS:1 and 9 also are secretin receptor-like GPCR polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of secretin receptor-like GPCR polynucleotides which encode biologically active secretin receptor-like GPCR polypeptides also are secretin receptor-like GPCR polynucleotides.

20
25

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the secretin receptor-like GPCR polynucleotides described above also are secretin receptor-like GPCR polynucleotides. Typically, homologous

30

secretin receptor-like GPCR polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known secretin receptor-like GPCR polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 μ C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the secretin receptor-like GPCR polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of secretin receptor-like GPCR polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 $^{\circ}$ C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973). Variants of human secretin-like GPCR polynucleotides or secretin receptor-like GPCR polynucleotides of other species can therefore be identified by hybridizing a putative homologous secretin receptor-like GPCR polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NOS:1 and 9 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to secretin receptor-like GPCR conditions also are secretin receptor-like GPCR polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example,

in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a secretin receptor-like GPCR polynucleotide having a nucleotide sequence shown in SEQ ID NOS:1 and 9 or the complement thereof and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):
$$T_m = 81.5\text{ }^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$
where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65 °C, or 50% formamide, 4X SSC at 42 °C, or 0.5X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2X SSC at 65 °C.

Preparation of Polynucleotides

A naturally occurring secretin receptor-like GPCR polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated GPCR polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises secretin receptor-like GPCR nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Human secretin receptor-like GPCR cDNA molecules can be made with standard molecular biology techniques, using secretin receptor-like GPCR mRNA as a template. Human secretin receptor-like GPCR cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize secretin receptor-like GPCR polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a secretin receptor-like GPCR polypeptide having, for example, an amino acid sequence shown in SEQ ID NOS:2 and 10 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences disclosed herein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic.* 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res.* 16, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer

Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72 °C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device

camera. Output/light intensity can be converted to electrical signal using appropriate software (e.g. GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable
5 for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

10 Human secretin receptor-like GPCR polypeptides can be obtained, for example, by purification from human cells, by expression of secretin receptor-like GPCR polynucleotides, or by direct chemical synthesis.

Protein Purification

15 Human secretin receptor-like GPCR polypeptides can be purified from any human cell which expresses the receptor, including host cells which have been transfected with secretin receptor-like GPCR polynucleotides. A purified secretin receptor-like GPCR polypeptide is separated from other compounds which normally associate
20 with the secretin receptor-like GPCR polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

25 Human secretin receptor-like GPCR polypeptide can be conveniently isolated as a complex with its associated G protein, as described in the specific examples, below.
30 pure; preferably, the preparations are 90% pure or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

5 To express secretin receptor-like GPCR polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding secretin receptor-like GPCR polypeptides and appropriate transcriptional and translational control elements. These methods 10 include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

15 A variety of expression vector/host systems can be utilized to contain and express sequences encoding a secretin receptor-like GPCR polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors 20 (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

25 The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in 30 bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life

Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In
5 mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding secretin receptor-like GPCR polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

10 Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the secretin receptor-like GPCR polypeptide. For example, when a large quantity of a secretin receptor-like GPCR polypeptide is needed for the
15 induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the secretin receptor-like GPCR polypeptide can be ligated into the vector in frame with sequences for the
20 amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem.* 264, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells
25 by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of

30 In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used.

For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol.* 153, 516-544, 1987.

Plant and Insect Expression Systems

5

If plant expression vectors are used, the expression of sequences encoding secretin receptor-like GPCR polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV
10 (Takamatsu, *EMBO J.* 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J.* 3, 1671-1680, 1984; Broglie *et al.*, *Science* 224, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ.* 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection.
15 Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express secretin receptor-like GPCR
20 polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding secretin receptor-like GPCR polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin
25 promoter. Successful insertion of secretin receptor-like GPCR polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which secretin receptor-like GPCR polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci.* 91, 3224-3227, 1994).

30

Mammalian Expression Systems

A number of viral-based expression systems can be used to express secretin receptor-like GPCR polypeptides in mammalian host cells. For example, if an adenovirus is
5 used as an expression vector, sequences encoding secretin receptor-like GPCR polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a secretin receptor-like GPCR polypeptide in infected
10 host cells (Logan & Shenk, *Proc. Natl. Acad. Sci.* 81, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments
15 of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (e.g., liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of
20 sequences encoding secretin receptor-like GPCR polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a secretin receptor-like GPCR polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases
25 where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of
various origins, both natural and synthetic. The efficiency of expression can be
30 enhanced by the inclusion of enhancers which are appropriate for the particular cell

system which is used (see Scharf *et al.*, *Results Probl. Cell Differ.* 20, 125-162, 1994).

Host Cells

5

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed secretin receptor-like GPCR polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

15

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express secretin receptor-like GPCR polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced secretin receptor-like GPCR sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, *ANIMAL CELL CULTURE*, R.I. Freshney, ed., 1986.

20

25

30

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell* 11, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell* 22, 817-23, 1980) genes which can be employed in *tk⁻* or *aprt⁻* cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci.* 77, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol.* 150, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci.* 85, 8047-51, 1988). Visible markers such as anthocyanins, β -glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol.* 55, 121-131, 1995).

Detecting Expression

Although the presence of marker gene expression suggests that the secretin receptor-like GPCR polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a secretin receptor-like GPCR polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a secretin receptor-like GPCR polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a secretin receptor-like GPCR polypeptide

induction or selection usually indicates expression of the GPCR polynucleotide

Alternatively, host cells which contain a secretin receptor-like GPCR polynucleotide and which express a secretin receptor-like GPCR polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a secretin receptor-like GPCR polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a secretin receptor-like GPCR polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a secretin receptor-like GPCR polypeptide to detect transformants which contain a secretin receptor-like GPCR polynucleotide.

A variety of protocols for detecting and measuring the expression of a secretin receptor-like GPCR polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a secretin receptor-like GPCR polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med.* 158, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding secretin receptor-like GPCR polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a secretin receptor-like GPCR

polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a secretin receptor-like GPCR polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode secretin receptor-like GPCR polypeptides can be designed to contain signal sequences which direct secretion of soluble secretin receptor-like GPCR polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound secretin receptor-like GPCR polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a secretin receptor-like GPCR polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as

those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the secretin receptor-like GPCR polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a secretin receptor-like GPCR polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif.* 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the secretin receptor-like GPCR polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol.* 12, 441-453, 1993.

Chemical Synthesis

Sequences encoding a secretin receptor-like GPCR polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al.* *Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a secretin receptor-like GPCR polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc.* 85, 2149-2154, 1963; Roberge *et al.*, *Science* 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of secretin receptor-like GPCR polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, *PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES*, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic secretin receptor-like GPCR polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation

procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the secretin receptor-like GPCR polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

5

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce secretin receptor-like GPCR polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

15

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter secretin receptor-like GPCR polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

25

Antibodies

30 includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, F(ab')₂, and Fv, which are capable of binding an epitope of a secretin receptor-like

GPCR polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

- 5 An antibody which specifically binds to an epitope of a secretin receptor-like GPCR polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity.
- 10 Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.
- 15 Typically, an antibody which specifically binds to a secretin receptor-like GPCR polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to secretin receptor-like GPCR polypeptides do not detect other proteins in immunochemical assays and can
- 20 immunoprecipitate a secretin receptor-like GPCR polypeptide from solution.

- Human secretin receptor-like GPCR polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a secretin receptor-like GPCR polypeptide can be
- 25 conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used
- 30

in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

5 Monoclonal antibodies which specifically bind to a secretin receptor-like GPCR polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature* 256, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods* 81, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci.* 80, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol.* 62, 109-120, 1984).

15 In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci.* 81, 6851-6855, 1984; Neuberger *et al.*, *Nature* 312, 604-608, 1984; Takeda *et al.*, *Nature* 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or
20 may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in
25 GB2188638B. Antibodies which specifically bind to a secretin receptor-like GPCR polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

30 Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to secretin receptor-like GPCR polypeptides. Antibodies with

related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobulin libraries (Burton, *Proc. Natl. Acad. Sci.* 88, 11120-23, 1991).

5 Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol.* 15,
10 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem.* 269, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using
15 standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer* 61, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165, 81-91).

20 Antibodies which specifically bind to secretin receptor-like GPCR polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci.* 86, 3833-3837, 1989;
25 Winter *et al.*, *Nature* 349, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and
30 which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a secretin receptor-like GPCR polypeptide is bound. The bound antibodies
5 can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a
10 specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45,
or 50 or more nucleotides long. Longer sequences also can be used. Antisense
15 oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of secretin receptor-like GPCR gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a
20 combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate,
25 carboxymethyl esters, carbonates, and phosphate triesters. *See Brown, Meth. Mol. Biol. 20, 1-8, 1994; Sonveaux, Meth. Mol. Biol. 26, 1-72, 1994; Uhlmann et al., Chem. Rev. 90, 543-583, 1990.*

Modifications of secretin receptor-like GPCR gene expression can be obtained by
30 designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the secretin receptor-like GPCR gene. Oligonucleotides

derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a secretin receptor-like GPCR polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a secretin receptor-like GPCR polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent secretin receptor-like GPCR nucleotides, can provide sufficient targeting specificity for secretin receptor-like GPCR mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular secretin receptor-like GPCR polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a secretin receptor-like GPCR polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or

a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. See, e.g., Agrawal *et al.*, *Trends Biotechnol.* 10, 152-158, 1992; Uhlmann *et al.*,
5 *Chem. Rev.* 90, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett.* 215, 3539-3542, 1987.

Ribozymes

10 Ribozymes are RNA molecules with catalytic activity. See, e.g., Cech, *Science* 236, 1532-1539; 1987; Cech, *Ann. Rev. Biochem.* 59, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol.* 2, 605-609; 1992, Couture & Stinchcomb, *Trends Genet.* 12, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (e.g., Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism
15 of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

20 The coding sequence of a secretin receptor-like GPCR polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the secretin receptor-like GPCR polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence
25 specific manner have been developed and described in the art (see Haseloff *et al.* *Nature* 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the
30 mRNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a secretin receptor-like GPCR RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate secretin receptor-like GPCR RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease secretin receptor-like GPCR expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human secretin-like GPCR. Such genes may represent genes which are
5 differentially expressed in disorders including, but not limited to, urinary incontinence, benign prostate hyperplasia, obesity and diseases related to obesity, cancer, diabetes, osteoporosis, anxiety, depression, hypertension, migraine, compulsive disorders, schizophrenia, autism, neurodegenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, and cancer
10 chemotherapy-induced vomiting. Further, such genes may represent genes which are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression
15 modulated under control versus experimental conditions. In addition, the human secretin-like GPCR gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only
20 be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is
25 isolated from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique which does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel

et al., ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

5

Transcripts within the collected RNA samples which represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 85, 208-12, 1988), subtractive hybridization (Hedrick *et al.*,
10 *Nature* 308, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 88, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science* 257, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the
15 treatment of disorders involving the human secretin-like GPCR. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human secretin-like GPCR. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human secretin-like GPCR gene
20 or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides assays for screening test compounds which bind to or
25 modulate the activity of a secretin receptor-like GPCR polypeptide or a secretin receptor-like GPCR polynucleotide. A test compound preferably binds to a secretin receptor-like GPCR polypeptide or polynucleotide. More preferably, a test compound decreases or increases the effect of secretin or a secretin analog as mediated via human secretin-like GPCR by at least about 10, preferably about 50,
30 more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. See Lam, *Anticancer Drug Des.* 12, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (see, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 6909, 1993; Erb *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 91, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37, 2678, 1994; Cho *et al.*, *Science* 261, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33, 2061; Gallop *et al.*, *J. Med. Chem.* 37, 1233, 1994). Libraries of compounds can be presented in solution (see, e.g., Houghten, *BioTechniques* 13, 412-421, 1992), or on beads (Lam, *Nature* 354, 82-84, 1991), chips (Fodor, *Nature* 364, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89, 1865-1869, 1992), or phage (Scott & Smith, *Science* 249, 386-390, 1990; Devlin, *Science* 249, 101-106, 1990; Gwiloa *et al.* *Proc. Natl. Acad. Sci.* 97, 6378-6382

High Throughput Screening

Test compounds can be screened for the ability to bind to secretin receptor-like GPCR polypeptides or polynucleotides or to affect secretin receptor-like GPCR activity or secretin receptor-like GPCR gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μ l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 19, 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity* 2, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

5

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

10

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to the secretin receptor-like GPCR polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, secretin and secretin analogs, as well as the natural ligands of known GPCRs and analogs or derivatives thereof.

20

In binding assays, either the test compound or the secretin receptor-like GPCR polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the secretin receptor-like GPCR polypeptide can then be accomplished, for example, by conversion of an appropriate substrate to a detectable product.

25

30

Alternatively, binding of a test compound to a secretin receptor-like GPCR polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a secretin receptor-like GPCR polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and secretin receptor-like GPCR polypeptide (McConnell *et al.*, *Science* 257, 1906-1912, 1992).

Determining the ability of a test compound to bind to a secretin receptor-like GPCR polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem.* 63, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a secretin receptor-like GPCR polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell* 72, 223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268, 12046-12054, 1993; Bartel *et al.*, *BioTechniques* 14, 920-924, 1993; Iwabuchi *et al.*, *Oncogene* 8, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the secretin receptor-like GPCR polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a secretin receptor-like GPCR polypeptide can be fused to a polynucleotide

encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the secretin receptor-like GPCR polypeptide.

It may be desirable to immobilize either the secretin receptor-like GPCR polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the secretin receptor-like GPCR polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the secretin receptor-like GPCR polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a solid support can be carried out in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the secretin receptor-like GPCR polypeptide is a fusion protein comprising a domain that allows the secretin receptor-like GPCR polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed secretin receptor-like GPCR polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a secretin receptor-like GPCR polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated secretin receptor-like GPCR polypeptides (or polynucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a secretin receptor-like GPCR polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the secretin receptor-like GPCR polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the secretin receptor-like GPCR polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the

secretin receptor-like GPCR polypeptide, and SDS gel electrophoresis under non-reducing conditions.

5 Screening for test compounds which bind to a secretin receptor-like GPCR polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a secretin receptor-like GPCR polypeptide or polynucleotide can be used in a cell-based assay system. A secretin receptor-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a secretin receptor-like GPCR
10 polypeptide or polynucleotide is determined as described above.

Functional Assays

15 Test compounds can be tested for the ability to increase or decrease a biological effect of a secretin receptor-like GPCR polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified secretin receptor-like GPCR polypeptide, a cell membrane preparation, or an intact cell with a test compound. A test compound which decreases a functional activity of a secretin
20 receptor-like GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing secretin receptor-like GPCR activity. A test compound which increases secretin receptor-like GPCR activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing GPCR activity.

25 One such screening procedure involves the use of melanophores which are transfected to express a secretin receptor-like GPCR polypeptide. Such a screening procedure may be employed for screening for a compound which inhibits
30 such an assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide by contacting the melanophore cells which comprise the receptor with both the receptor ligand (e.g., secretin or a secretin

analog) and a test compound to be screened. Inhibition of the signal generated by the ligand indicates that a test compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a test compound which activates the receptor by contacting such cells with compounds to be screened and determining whether each test compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a human secretin-like GPCR polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation (*see, e.g., Science* 246, 181-296, 1989). For example, test compounds may be contacted with a cell which expresses a human secretin-like GPCR polypeptide and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the test compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding a human secretin-like GPCR polypeptide into *Xenopus* oocytes to transiently express the receptor. The transfected oocytes can then be contacted with the receptor ligand and a test compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for test compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing a human secretin-like GPCR polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Details of functional assays such as those described above are provided in the specific examples, below.

Gene Expression

In another embodiment, test compounds which increase or decrease secretin receptor-like GPCR gene expression are identified. A secretin receptor-like GPCR polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the secretin receptor-like GPCR polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of secretin receptor-like GPCR mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a secretin receptor-like GPCR polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a secretin receptor-like GPCR polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell-based assay system. The secretin receptor-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as

those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

5

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a secretin receptor-like GPCR polypeptide, secretin receptor-like GPCR polynucleotide, antibodies which specifically bind to a secretin receptor-like GPCR polypeptide, or mimetics, agonists, antagonists, or inhibitors of a secretin receptor-like GPCR polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

15

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

20

25

30

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in the form of aqueous solutions, suspensions, emulsions, or sterile powders for reconstitution. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, sus-

pensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

GPCRs are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate a GPCR on the one hand and which can inhibit the function of a GPCR on the other hand. For example, compounds which activate a GPCR may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate GPCRs are useful in treating various cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

In general, compounds which inhibit activation of a GPCR can be used for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit GPCRs also are useful in reversing endogenous anorexia, in the control of bulimia, and in treating various cardiovascular ailments such as caused by excessive pulmonary blood flow or hypotension. In particular, regulation of GPCR can be used to treat anxiety, depression, hypertension, migraine, compulsive disorders, schizophrenia, autism, neurodegenerative disorders, such as Alzheimer's disease, Parkinsonism, and Huntington's chorea, and cancer disorders involving regulation of body temperature and blood pressure.

Urinary incontinence. This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating urinary incontinence (UI). Urinary incontinence is the involuntary loss of urine. Urge urinary incontinence (UUI) is one of the most common types of UI together with stress urinary incontinence (SUI) which is usually caused by a defect in the urethral closure mechanism. UUI is often associated with neurological disorders or diseases causing neuronal damages such as dementia, Parkinson's disease, multiple sclerosis, stroke and diabetes, although it also occurs in individuals with no such disorders. One of the usual causes of UUI is overactive bladder (OAB) which is a medical condition referring to the symptoms of frequency and urgency derived from abnormal contractions and instability of the detrusor muscle.

There are several medications for urinary incontinence on the market today mainly to help treating UUI. Therapy for OAB is focused on drugs that affect peripheral neural control mechanisms or those that act directly on bladder detrusor smooth muscle contraction, with a major emphasis on development of anticholinergic agents. These agents can inhibit the parasympathetic nerves which control bladder voiding or can exert a direct spasmolytic effect on the detrusor muscle of the bladder. This results in a decrease in intravesicular pressure, an increase in capacity and a reduction in the frequency of bladder contraction. Orally active anticholinergic drugs such as propantheline (ProBanthine), tolterodine tartrate (Detrol) and oxybutynin (Ditropan) are the most commonly prescribed drugs. However, their most serious drawbacks are unacceptable side effects such as dry mouth, abnormal visions, constipation, and central nervous system disturbances. These side effects lead to poor compliance. Dry mouth symptoms alone are responsible for a 70% non-compliance rate with oxybutynin. The inadequacies of present therapies highlight the need for novel, efficacious, safe, orally available drugs that have fewer side effects.

Benign prostatic hyperplasia: This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating venign prostatic hyperplasia. Benign prostatic hyperplasia (BPH) is the benign

nodular hyperplasia of the periurethral prostate gland commonly seen in men over the age of 50. The overgrowth occurs in the central area of the prostate called the transition zone, which wraps around the urethra. BPH causes variable degrees of bladder outlet obstruction resulting in progressive lower urinary tract syndromes (LUTS) characterized by urinary frequency, urgency, and nocturia due to incomplete emptying and rapid refilling of the bladder. The actual cause of BPH is unknown but may involve age-related alterations in balance of steroidal sex hormones.

The selective $\alpha 1$ -adrenoceptor antagonists, such as prazosin, indoramin and tamsulosin are used as an adjunct in the symptomatic treatment of urinary obstruction caused by BPH, although they do not affect on the underlying cause of BPH. In BPH, increased sympathetic tone exacerbates the degree of obstruction of the urethra through contraction of prostatic and urethral smooth muscle. These compounds inhibit sympathetic activity, thereby relaxing the smooth muscle of the urinary tract. Uroselective $\alpha 1$ -antagonists and $\alpha 1$ -antagonists with high tissue selectivity for lower urinary tract smooth muscle that do not provoke hypotensive side-effects should be developed for the treatment.

Drugs blocking dihydrotestosterone have been used to reduce the size of the prostate. 5α -reductase inhibitors such as finasteride are prescribed for BPH. These agents selectively inhibit 5α -reductase which mediates conversion of testosterone to dihydrotestosterone, thereby reducing plasma dihydrotestosterone levels and thus prostate growth. The 5α -reductase inhibitors do not bind to androgen receptors and do not affect testosterone levels nor do they possess feminizing side-effects.

Androgen receptor antagonists are used for the treatment of prostatic hyperplasia due to excessive action or production of testosterone. Various antiandrogens are under

activity, orally active aromatase inhibitors, luteinizing hormone-releasing hormone (LHRH) analogues.

Obesity. This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

This gene, translated proteins and agents which modulate this gene or portions of the gene or its products also are useful for treating obesity/overweight-associated comorbidities including hypertension, type 2 diabetes, coronary artery disease, hyperlipidemia, stroke, gallbladder disease, gout, osteoarthritis, sleep apnea and respiratory problems, some types of cancer including endometrial, breast, prostate and colon cancer, thrombotic disease, polycystic ovarian syndrome; reduced fertility, complications of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and depression.

Cancer. Human GPCRs provide a potential target for treating cancer. Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled

cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target

and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and

toxicological analyses form the basis for drug development and subsequent testing in humans.

5 Diabetes. Diabetes also can be potentially treated by regulating the activity of human secretin-like GPCR. Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset) that results from a loss of cells which make and secrete insulin, and type 2
10 diabetes (adult onset) which is caused by a defect in insulin secretion and a defect in insulin action.

Type 1 diabetes is initiated by an autoimmune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction
15 from occurring or that stop the reaction before destruction of the beta cells has been accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration are also potential therapies.

20 Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properly detect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this
25 disease.

The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulin receptor in muscle, liver and fat will cause a decrease in blood glucose and a normalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate
30 the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellular end process, *i.e.* glucose transport or

various enzyme systems, to generate an insulin-like effect and therefore a produce beneficial outcome. Because overweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

Both Type I and Type diabetes can be treated with agents that mimic insulin action or that treat diabetic complications by reducing blood glucose levels. Likewise agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

10 *Osteoporosis*. Osteoporosis, too, can potentially be treated by regulating human secretin-like GPCR. Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. It is the most common human metabolic bone disorder. Established osteoporosis includes the presence of fractures.

15 Bone turnover occurs by the action of two major effector cell types within bone: the osteoclast, which is responsible for bone resorption, and the osteoblast, which synthesizes and mineralizes bone matrix. The actions of osteoclasts and osteoblasts are highly coordinated. Osteoclast precursors are recruited to the site of turnover;
20 they differentiate and fuse to form mature osteoclasts which then resorb bone. Attached to the bone surface, osteoclasts produce an acidic microenvironment in a tightly defined junction between the specialized osteoclast border membrane and the bone matrix, thus allowing the localized solubilization of bone matrix. This in turn facilitate the proteolysis of demineralized bone collagen. Matrix degradation is
25 thought to release matrix-associated growth factor and cytokines, which recruit osteoblasts in a temporally and spatially controlled fashion. Osteoblasts synthesize and secrete new bone matrix proteins, and subsequently mineralize this new matrix.

change in bone mass. In pathological states, such as osteoporosis, the balance
30 between resorption and formation is altered such that bone loss occurs. See WO
99/45973.

The osteoclast itself is the direct or indirect target of all currently available osteoporosis agents with the possible exception of fluoride. Antiresorptive therapy prevents further bone loss in treated individuals. Osteoblasts are derived from multipotent stem cells which reside in bone marrow and also gives rise to adipocytes, chondrocytes, fibroblasts and muscle cells. Selective enhancement of osteoblast activity is a highly desirable goal for osteoporosis therapy since it would result in an increase in bone mass, rather than a prevention of further bone loss. An effective anabolic therapy would be expected to lead to a significantly greater reduction in fracture risk than currently available treatments.

The agonists or antagonists to the newly discovered polypeptides may act as antiresorptive by directly altering the osteoclast differentiation, osteoclast adhesion to the bone matrix or osteoclast function of degrading the bone matrix. The agonists or antagonists could indirectly alter the osteoclast function by interfering in the synthesis and/or modification of effector molecules of osteoclast differentiation or function such as cytokines, peptide or steroid hormones, proteases, etc.

The agonists or antagonists to the newly discovered polypeptides may act as anabolics by directly enhancing the osteoblast differentiation and /or its bone matrix forming function. The agonists or antagonists could also indirectly alter the osteoblast function by enhancing the synthesis of growth factors, peptide or steroid hormones or decreasing the synthesis of inhibitory molecules.

The agonists and antagonists may be used to mimic, augment or inhibit the action of the newly discovered polypeptides which may be useful to treat osteoporosis, Paget's disease, degradation of bone implants particularly dental implants.

Asthma Allergy is a complex process in which environmental antigens induce clinically adverse reactions. The inducing antigens, called allergens, typically elicit a specific IgE response and, although in most cases the allergens themselves have little

or no intrinsic toxicity, they induce pathology when the IgE response in turn elicits an IgE-dependent or T cell-dependent hypersensitivity reaction. Hypersensitivity reactions can be local or systemic and typically occur within minutes of allergen exposure in individuals who have previously been sensitized to an allergen. The hypersensitivity reaction of allergy develops when the allergen is recognized by IgE antibodies bound to specific receptors on the surface of effector cells, such as mast cells, basophils, or eosinophils, which causes the activation of the effector cells and the release of mediators that produce the acute signs and symptoms of the reactions. Allergic diseases include asthma, allergic rhinitis (hay fever), atopic dermatitis, and anaphylaxis.

Asthma is thought to arise as a result of interactions between multiple genetic and environmental factors and is characterized by three major features: 1) intermittent and reversible airway obstruction caused by bronchoconstriction, increased mucus production, and thickening of the walls of the airways that leads to a narrowing of the airways, 2) airway hyperresponsiveness caused by a decreased control of airway caliber, and 3) airway inflammation. Certain cells are critical to the inflammatory reaction of asthma and they include T cells and antigen presenting cells, B cells that produce IgE, and mast cells, basophils, eosinophils, and other cells that bind IgE. These effector cells accumulate at the site of allergic reaction in the airways and release toxic products that contribute to the acute pathology and eventually to the tissue destruction related to the disorder. Other resident cells, such as smooth muscle cells, lung epithelial cells, mucus-producing cells, and nerve cells may also be abnormal in individuals with asthma and may contribute to the pathology. While the airway obstruction of asthma, presenting clinically as an intermittent wheeze and shortness of breath, is generally the most pressing symptom of the disease requiring immediate treatment, the inflammation and tissue destruction associated with the

causative disorder requiring long-term management.

Despite recent important advances in our understanding of the pathophysiology of asthma, the disease appears to be increasing in prevalence and severity (Gergen and Weiss, *Am. Rev. Respir. Dis.* 146, 823-24, 1992). It is estimated that 30-40% of the population suffer with atopic allergy, and 15% of children and 5% of adults in the population suffer from asthma (Gergen and Weiss, 1992). Thus, an enormous burden is placed on our health care resources. However, both diagnosis and treatment of asthma are difficult. The severity of lung tissue inflammation is not easy to measure and the symptoms of the disease are often indistinguishable from those of respiratory infections, chronic respiratory inflammatory disorders, allergic rhinitis, or other respiratory disorders. Often, the inciting allergen cannot be determined, making removal of the causative environmental agent difficult. Current pharmacological treatments suffer their own set of disadvantages. Commonly used therapeutic agents, such as beta agonists, can act as symptom relievers to transiently improve pulmonary function, but do not affect the underlying inflammation. Agents that can reduce the underlying inflammation, such as anti-inflammatory steroids, can have major drawbacks that range from immunosuppression to bone loss (Goodman and Gilman's THE PHARMACOLOGIC BASIS OF THERAPEUTICS, Seventh Edition, MacMillan Publishing Company, NY, USA, 1985). In addition, many of the present therapies, such as inhaled corticosteroids, are short-lasting, inconvenient to use, and must be used often on a regular basis, in some cases for life, making failure of patients to comply with the treatment a major problem and thereby reducing their effectiveness as a treatment.

Because of the problems associated with conventional therapies, alternative treatment strategies have been evaluated. Glycophorin A (Chu and Sharom, *Cell. Immunol.* 145, 223-39, 1992), cyclosporin (Alexander *et al.*, *Lancet* 339, 324-28, 1992), and a nonapeptide fragment of IL-2 (Zav'yalov *et al.*, *Immunol. Lett.* 31, 285-88, 1992) all inhibit interleukin-2 dependent T lymphocyte proliferation; however, they are known to have many other effects. For example, cyclosporin is used as an immunosuppressant after organ transplantation. While these agents may represent alternatives to steroids in the treatment of asthmatics, they inhibit interleukin-2

dependent T lymphocyte proliferation and potentially critical immune functions associated with homeostasis. Other treatments that block the release or activity of mediators of bronchoconstriction, such as cromones or anti-leukotrienes, have recently been introduced for the treatment of mild asthma, but they are expensive and not effective in all patients and it is unclear whether they have any effect on the chronic changes associated with asthmatic inflammation. What is needed in the art is the identification of a treatment that can act in pathways critical to the development of asthma that both blocks the episodic attacks of the disorder and preferentially dampens the hyperactive allergic immune response without immunocompromising the patient.

Many of the mediators involved in airway smooth muscle contraction and in the chemoattraction of inflammatory cells exert their effects through GPCR binding. Among the mediators of smooth muscle contraction are leukotrienes, platelet-activating factor, endothelin-1, adenosine, and thromboxane A₂. Receptor antagonists that block the activation of GPCRs by some of these mediators have been successfully used as treatments for asthma. Among the chemoattractants of inflammatory cells are the chemokines, such as eotaxin, MCP-4, RANTES, and IL-8. Chemokine receptor antagonists similarly are being developed as treatments for asthma. Sarau *et al.*, *Mol. Pharmacol.* 56, 657-63, 1999; Kitaoura *et al.*, *J. Biol. Chem.* 271, 7725-30, 1996; Liggett *et al.*, *Am. J. Respir. Crit. Care Med.* 152, 394-402, 1995; Panettieri *et al.*, *J. Immunol.* 154, 2358-65, 1995; Noveral *et al.*, *Am. J. Physiol.* 263, L317-24, 1992; Honda *et al.*, *Nature* 349, 342-46, 1991.

Activation of some GPCRs may conversely have beneficial effects in asthma. For example, receptor agonists that activate the β 1- and β 2-adrenergic GPCRs are used therapeutically to relax contracted airway smooth muscle in the treatment of asthma.

Receptor antagonists may play an important role in the treatment of asthma.

Cardiovascular Diseases. Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

5

Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement of the metabolizing tissue. It includes all forms of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

10

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

15

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

20

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

25

Hypertensive vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications.

30

Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

CNS Disorders. CNS disorders which may be treated include brain injuries, cerebrovascular diseases and their consequences, Parkinson's disease, corticobasal degeneration, motor neuron disease, dementia, including ALS, multiple sclerosis, traumatic brain injury, stroke, post-stroke, post-traumatic brain injury, and small-vessel cerebrovascular disease. Dementias, such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinsonism linked to chromosome 17, frontotemporal dementias, including Pick's disease, progressive nuclear palsy, corticobasal degeneration, Huntington's disease, thalamic degeneration, Creutzfeld-Jakob dementia, HIV dementia, schizophrenia with dementia, and Korsakoff's psychosis also can be treated. Similarly, it may be possible to treat cognitive-related disorders, such as mild cognitive impairment, age-associated memory impairment, age-related cognitive decline, vascular cognitive impairment, attention deficit disorders, attention deficit hyperactivity disorders, and memory disturbances in children with learning disabilities, by regulating the activity of human secretin-like GPCR.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (e.g., a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or secretin receptor-agonist) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention

pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

5 A reagent which affects secretin receptor-like GPCR activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce secretin receptor-like GPCR activity. The reagent preferably binds to an expression product of a human secretin-like GPCR gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can
10 then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about
15 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain,
20 lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5
25 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and
30 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al.* *Trends in Biotechnol.* 11, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem.* 263, 621-24 (1988); Wu *et al.*, *J. Biol. Chem.* 269, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem.* 266, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of one of ordinary skill in the art. The therapeutically effective dose of an active ingredient which increases or decreases secretin receptor-like GPCR activity relative to the secretin receptor-like GPCR activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50} .

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total

dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

A reagent reduces expression of a secretin receptor-like GPCR gene or the activity of a secretin receptor-like GPCR polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the

reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a secretin receptor-like GPCR gene or the activity of a secretin receptor-like GPCR polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to secretin receptor-like GPCR-specific mRNA, quantitative RT-PCR, immunologic detection of a secretin receptor-like GPCR polypeptide, or measurement of secretin receptor-like GPCR activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

20

Diagnostic Methods

GPCRs also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode a GPCR. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

30

Differences can be determined between the cDNA or genomic sequence encoding a secretin receptor-like GPCR in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g., Myers et al., Science 230, 1242, 1985*). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g., Cotton et al., Proc. Natl. Acad. Sci. USA 85, 4397-4401, 1985*). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA

In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

- 71 -

Altered levels of a secretin receptor-like GPCR also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1*Detection of secretin receptor-like GPCR activity*

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4
5 and the expression vector pCEV4-secretin receptor-like GPCR polypeptide obtained
is transfected into human embryonic kidney 293 cells. From these cells extracts are
obtained and centrifuged at 1000 rpm for 5 minutes at 4 °C. The supernatant is
centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding
10 buffer containing 50 mM Tris HCl, 5 mM MgSO₄, 1 mM EDTA, 100 mM NaCl, pH
7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10
µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the
protein concentration required to bind less than 10% of the added radioligand, i.e.
secretin, are added to 96-well polypropylene microtiter plates containing ¹²⁵I-labeled
15 ligand or test compound, non-labeled peptides, and binding buffer to a final volume
of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the
presence of increasing concentrations (0.1 nM to 4 nM) of ¹²⁵I-labeled ligand or test
20 compound (specific activity 2200 Ci/mmol). The binding affinities of different test
compounds are determined in equilibrium competition binding assays, using 0.1 nM
¹²⁵I-peptide in the presence of twelve different concentrations of each test compound.

Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is
stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using
25 a cell harvester. Radioactivity is measured by scintillation counting, and data are
analyzed by a computerized non-linear regression program.

Incubation of membrane protein in the presence of 100 nM of unlabeled peptide.
30 Protein concentration is measured by the Bradford method using Bio-Rad Reagent,

with bovine serum albumin as a standard. It is shown that the polypeptide of SEQ ID NO: 2 has a secretin receptor-like GPCR activity.

EXAMPLE 2

5 *Radioligand binding assays*

Human embryonic kidney 293 cells transfected with a polynucleotide which expresses human secretin-like GPCR are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged
10 at 1000 rpm for 5 minutes at 4 °C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4 °C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM MgSO₄, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration
15 required to bind less than 10% of the added radioligand, i.e. secretin, are added to 96-well polypropylene microtiter plates containing ¹²⁵I-labeled ligand or test compound, non-labeled peptides, and binding buffer to a final volume of 250 µl.

In equilibrium saturation binding assays, membrane preparations are incubated in the
20 presence of increasing concentrations (0.1 nM to 4 nM) of ¹²⁵I-labeled ligand or test compound (specific activity 2200 Ci/mmol). The binding affinities of different test compounds are determined in equilibrium competition binding assays, using 0.1 nM ¹²⁵I- peptide in the presence of twelve different concentrations of each test compound.

25 Binding reaction mixtures are incubated for one hour at 30 °C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

30

Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. A test compound which increases the radioactivity of membrane protein by at least 15% relative to radioactivity of membrane protein which was not incubated with a test compound is identified as a compound which binds to a human secretin-like GPCR polypeptide.

EXAMPLE 3

Effect of a test compound on human secretin-like GPCR -mediated cyclic AMP formation

Receptor-mediated inhibition of cAMP formation can be assayed in host cells which express human secretin-like GPCR. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 µg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 µg/ml phosphoramidon for 20 minutes at 37 °C in 5% CO₂. A test compound is added and incubated for an additional 10 minutes at 37 °C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4 °C for 15 minutes. cAMP content in the stopping solution is measured by radioimmunoassay.

Radioactivity is quantified using a gamma counter equipped with data reduction software. A test compound which decreases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential inhibitor of cAMP formation. A test compound which increases radioactivity of the contents of a well relative to radioactivity of the

enhancer of cAMP formation.

EXAMPLE 4*Effect of a test compound on the mobilization of intracellular calcium*

Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al.*, *J. Neurochem.* 57, 562-74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS, incubated with a test compound, and loaded with 100 μ l of Fura-2/AM (10 μ M) for 20-40 minutes. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nm, with excitation wavelengths alternating between 340 nm and 380 nm. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

EXAMPLE 5*Effect of a test compound on phosphoinositide metabolism*

Cells which stably express human secretin-like GPCR cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 μ l of medium containing 1% serum and 0.5 μ Ci 3 H-myo-inositol. The plates are incubated overnight in a CO₂ incubator (5% CO₂ at 37 °C). Immediately before the assay, the medium is removed and replaced by 200 μ l of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium for 20 minutes. During this interval, cells also are equilibrated with antagonist, added as a 10 μ l aliquot of a 20-fold concentrated solution in PBS.

5 The ^3H -inositol phosphate accumulation from inositol phospholipid metabolism is started by adding 10 μl of a solution containing a test compound. To the first well 10 μl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows.

10 The plates are incubated in a CO_2 incubator for one hour. The reaction is terminated by adding 15 μl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4 $^\circ\text{C}$. After neutralizing TCA with 40 μl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 μl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 μl of water, followed by 2 x 200 μl of 5 mM sodium tetraborate/60 mM ammonium formate.

20 The ^3H -IPs are eluted into empty 96-well plates with 200 μl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

EXAMPLE 6

Receptor Binding Methods

25 Standard Binding Assays. Binding assays are carried out in a binding buffer containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM MgCl_2 . The standard assay for radioligand binding to membrane fragments comprising secretin receptor-

30 Dynabead, Immulon II (Kodak) or plates. Radioligand is diluted in binding buffer (PMSF/Baci to the desired cpm per 50 μl , then 50 μl aliquots are added to the wells. For non-specific binding samples, 5 μl of 40 μM cold ligand also is added per well.

- 77 -

Binding is initiated by adding 150 μ l per well of membrane diluted to the desired concentration (10-30 μ g membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2
5 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 μ l of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when
10 free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethyleneimine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

- 15 1. Competitive radioligand binding assays with a concentration range of cold ligand vs. 125 I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 μ l each) are then added per microtiter well.
20 Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.
- 25 2. Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-linking of radioligand with BSA. The cross-linking step is carried out as described below.
- 30 3. Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are

also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 μ l, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are re-suspended in 200 μ l per microtiter plate well of ice-cold binding buffer without BSA. Then 5 μ l per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled proteins are visualized by autoradiography of the dried gels with Kodak XAR film and DuPont image intensifier screens.

EXAMPLE 8

Membrane Solubilization

Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM CaCl_2 (solubilization buffer). The highly soluble and zwittergent are made up in solubilization buffer at 10% concentrations and stored as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-

thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

EXAMPLE 9

Assay of Solubilized Receptors

After binding of ^{125}I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10 °C.).

1. Column chromatography (Knuhtsen *et al.*, *Biochem. J.* 254, 641-647, 1988). Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes and 1 mg/ml bovine serum albumin. Samples of solubilized membranes (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA* 69, 318-322, 1972). For a 100 µl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of 25% (w/v) polyethyleneglycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added

per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the phosphate buffer. PEG-precipitated receptor : 125 I-ligand complex is determined by gamma counting of the filters.

5

3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem.* 132, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 μ l) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : 125 I-ligand complex adsorbed to filters are determined by gamma counting.

10

4. Charcoal/Dextran (Paul and Said, *Peptides* 7[Suppl. 1],147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The suspension is stirred for 10 minutes at room temperature and then stored at 4°C. until use. To measure R:L complex, 4 parts by volume of charcoal/dextran suspension are added to 1 part by volume of solubilized membrane. The samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor : 125 I-ligand complexes remain in the supernatant and are determined by gamma counting.

15

20

25

EXAMPLE 10

- Binding of biotinyl-receptor to GH₄ C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol.

30

the binding incubations contain 10 nM Bio-S29. ^{125}I ligand is added as a tracer at levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

- 5 Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM MgCl_2 , to obtain 100,000 x g supernatants containing solubilized R:L complex.

- 10 Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 $^{\circ}\text{C}$. Then the mixture is applied to a column and the non-bound material is washed through. Binding of radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g
15 supernatant with that in the column effluent after adsorption to SA-agarose. Finally, the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

- 20 The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin +1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

- 25 Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is
30 pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5

mM $MgCl_2$, and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After
5 each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific contaminants. All these fractions are stored frozen at $-90^{\circ}C$.

EXAMPLE 11

Identification of test compounds that bind to secretin receptor-like GPCR polypeptides

15 Purified secretin receptor-like GPCR polypeptides comprising a glutathione-S- transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human secretin receptor-like GPCR polypeptides comprise an amino acid sequence shown in SEQ ID NOS:2 and 10. The test com-
20 pounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a secretin receptor-like GPCR polypeptide is detected
25 by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound is not incubated is identified as a compound which binds to

EXAMPLE 12

Identification of a test compound which decreases human secretin-like GPCR gene expression

5 A test compound is administered to a culture of human gastric cells and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

10 RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ³²P-labeled secretin receptor-like GPCR-specific probe at 65 °C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS:1 AND 10. A test compound which decreases the secretin receptor-like GPCR-specific signal relative to the signal
15 obtained in the absence of the test compound is identified as an inhibitor of secretin receptor-like GPCR gene expression.

EXAMPLE 13

Expression profiling

20 An expression profiling was done from the following tissues:

coronary smooth muscle cells, brain, testis, pancreas, stomach, cerebellum, trachea, adrenal gland, skeletal muscle, salivary gland, small intestine, prostata, fetal liver, placenta, fetal brain, uterus, mammary gland, heart, spleen, lung, HeLa cells, liver,
25 kidney, thymus, bone marrow, thyroid, colon, bladder, spinal cord, peripheral blood, liver liver cirrhosis, pancreas liver cirrhosis, spleen liver cirrhosis, total Alzheimer brain, fetal lung, breast tumor, colon tumor, lung tumor, HEK 293 cells, adipose, pericardium, fetal heart, thyroid tumor, MDA MB 231 cells, HEP G2 cells, HUVEC cells, fetal kidney, breast, Jurkat T-cells, Alzheimer brain cortex, cervix, esophagus,
30 thalamus, precentral gyrus, hippocampus, occipital lobe, cerebral peduncles, postcentral gyrus, temporal lobe, parietal lobe, cerebellum (right), cerebellum (left),

tonsilla cerebelli, cerebral meninges, pons, frontal lobe, cerebral cortex, corpus callosum, vermis cerebelli, Alzheimer brain frontal lobe, interventricular septum, heart atrium (right), heart atrium (left), heart ventricle (left)

5 Total cellular RNA was isolated from cells by one of two standard methods: 1) guanidine isothiocyanate/Cesium chloride density gradient centrifugation; or with the Tri-Reagent protocol according to the manufacturer's specifications (Molecular Research Center, Inc., Cincinnati, Ohio). Total RNA prepared by the Tri-reagent protocol was treated with DNase I to remove genomic DNA contamination.

10

For relative quantitation of the mRNA distribution of secretin receptor-like GPCR, total RNA from each cell or tissue source was first reverse transcribed. 85 µg of total RNA was reverse transcribed using 1 µmole random hexamer primers, 0.5 mM each of dATP, dCTP, dGTP and dTTP (Qiagen, Hilden, Germany), 3000 U RnaseQut (Invitrogen, Groningen, Netherlands) in a final volume of 680 µl. The first strand synthesis buffer and Omniscript (2 u/µl) reverse transcriptase were from (Qiagen, Hilden, Germany). The reaction was incubated at 37 degree. C. for 90 minutes and cooled on ice. The volume was adjusted to 6800 µl with water, yielding a final concentration of 12,5 ng/µl of starting RNA.

20

For relative quantitation of the distribution of secretin receptor-like GPCR mRNA in cells and tissues the Perkin Elmer ABI Prism.RTM. 7700 Sequence Detection system or Biorad iCycler was used according to the manufacturer's specifications and protocols. PCR reactions were set up to quantitate secretin receptor-like GPCR and the housekeeping genes HPRT, GAPDH, beta-actin and others Forward and reverse primers and probe for the secretin receptor-like GPCR were designed using the Perkin Elmer ABI Piimer Express.TM. software and were synthesized by

sequence was Primer1 (TCTGGACTACATCACCTGCATTGGGCTC). The secretin receptor
30 like GPCR reverse primer sequence was Primer2 (TCTGGACTACATCACCTGCATTGGGCTC). The fluorogenic probe, labelled

with FAM as the reporter dye and TAMRA as the quencher, is Probe1 (ACCAAGCTTAGGATTGAGACGC). The following reactions in a final volume of 25 μ l were set up : 1X TaqMan buffer A, 5.5 mM MgCl₂, 200 nM each of dATP, dCTP, dGTP and dUTP, 0.025 U/ μ l AmpliTaq Gold.TM., 0.01 U/ μ l AmpErase UNG.RTM. and probe 1X, secretin receptor-like GPCR forward and reverse primers each at 200 nM, 200 nM secretin receptor-like GPCR FAM/TAMRA-labelled probe, and 5 μ l of template cDNA. Thermal cycling parameters were 2 min HOLD at 50.degree. C., 10 min HOLD at 95.degree. C., followed by melting at 95.degree. C. for 15 sec and annealing/extending at 60.degree. C. for 1 min for each of 40 cycles.

Calculation of corrected CT values

The CT-value is calculated as described above. The CF-value is calculated as followed :

1. PCR reactions were set up to quantitate the housekeeping genes (HKG) for each cDNA sample.
2. CT_{HKG}-values were calculated as described above
3. CT-mean values of all HKG for each cDNA are calculated (n = number of HKG):

$$(CT_{HKG1}\text{-value} + CT_{HKG2}\text{-value} + CT_{HKG-X}\text{-value}) / n = CT_{cDNA-X}\text{-mean values}$$
 (n = number of HKG)
4. $(CT_{cDNA-1}\text{-mean value} + CT_{cDNA-X}\text{-mean value}) / y = CT_{panel}\text{-mean value}$
 (y = number of cDNAs)
5. $CT_{panel}\text{-mean value} - CT_{cDNA-X}\text{-mean value} = CF_{cDNA-X}$
6. $CT_{cDNA-X} + CF_{cDNA-X} = CT_{cor-cDNA-X}$

Calculation of relative expression

Definition : highest $CT_{\text{cor-cDNA-X}} \neq 40$ is defined as $CT_{\text{cor-cDNA-X}} [\text{high}]$

5 Relative Expression = $2^{e(CT_{\text{cor-cDNA-X}} [\text{high}] - CT_{\text{cor-cDNA-Y}})}$

The results of the mRNA-quantification (expression profiling) are shown in Figs. 17A, B and C.

10 It has been discovered by the present applicant that the secretin receptor-like GPCR is expressed in different human tissues. The receptor is highly expressed in total Alzheimer brain, Alzheimer brain cortex, Alzheimer brain frontal lobe, cerebellum, postcentral gyrus, cerebral meninges, coronary smooth muscle cells, lung tumor, liver (liver cirrhosis), HEK292, placenta.

15 The receptor is highly expressed in different brain tissues as total Alzheimer brain, Alzheimer brain cortex, postcentral gyrus and cerebral meninges. The expression in the above mentioned tissues suggests an association between secretin receptor-like GPCR and peripheral and central nervous system diseases.

20 The receptor is highly expressed in coronary smooth muscle cells. The expression in the above mentioned tissues suggests an accociation between secretin receptor-like GPCR and cardio-vascular diseases.

25 The receptor is highly expressed in lung tumor. The expression in the above mentioned tissues suggests an accociation between secretin receptor-like GPCR and cancer.

30 The receptor is highly expressed in liver, liver (liver cirrhosis), HEK292. The expression in the above mentioned tissues suggests an association between secretin receptor-like GPCR and diseases of the liver and kidney.

The receptor is highly expressed in placenta. The expression in the above mentioned tissues suggests an association between secretin receptor-like GPCR and genito-urinary diseases.

CLAIMS

1. An isolated polynucleotide encoding a secretin receptor-like GPCR polypeptide and being selected from the group consisting of:
- 5
- a) a polynucleotide encoding a secretin receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:
- 10
- amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 2;
- the amino acid sequence shown in SEQ ID NO: 2;
- amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO: 10; and
- the amino acid sequence shown in SEQ ID NO: 10.
- 15
- b) a polynucleotide comprising the sequence of SEQ ID NO: 1 or 9;
- c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);
- d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the
- 20
- degeneration of the genetic code; and
- e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).
- 25
2. An expression vector containing any polynucleotide of claim 1.
3. A host cell containing the expression vector of claim 2.

a polynucleotide of claim 1

5. A method for producing a secretin receptor-like GPCR polypeptide, wherein the method comprises the following steps:
- 5 a) culturing the host cell of claim 3 under conditions suitable for the expression of the secretin receptor-like GPCR polypeptide; and
- b) recovering the secretin receptor-like GPCR polypeptide from the host cell culture.
- 10 6. A method for detection of a polynucleotide encoding a secretin receptor-like GPCR polypeptide in a biological sample comprising the following steps:
- a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and
- b) detecting said hybridization complex.
- 15 7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.
8. A method for the detection of a polynucleotide of claim 1 or a secretin receptor-like GPCR polypeptide of claim 4 comprising the steps of:
- 20 contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the secretin receptor-like GPCR polypeptide.
9. A diagnostic kit for conducting the method of any one of claims 6 to 8.
- 25 10. A method of screening for agents which decrease the activity of a secretin receptor-like GPCR, comprising the steps of:
- contacting a test compound with any secretin receptor-like GPCR polypeptide encoded by any polynucleotide of claim 1;
- detecting binding of the test compound to the secretin receptor-like GPCR polypeptide, wherein a test compound which binds to the polypeptide is
- 30

identified as a potential therapeutic agent for decreasing the activity of a secretin receptor-like GPCR.

11. A method of screening for agents which regulate the activity of a secretin
5 receptor-like GPCR, comprising the steps of:
contacting a test compound with a secretin receptor-like GPCR polypeptide
encoded by any polynucleotide of claim 1; and
detecting a secretin receptor-like GPCR activity of the polypeptide, wherein a
test compound which increases the secretin receptor-like GPCR activity is
10 identified as a potential therapeutic agent for increasing the activity of the
secretin receptor-like GPCR, and wherein a test compound which decreases
the secretin receptor-like GPCR activity of the polypeptide is identified as a
potential therapeutic agent for decreasing the activity of the secretin receptor-
like GPCR.
12. A method of screening for agents which decrease the activity of a secretin
15 receptor-like GPCR, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting
20 binding of the test compound to the polynucleotide, wherein a test compound
which binds to the polynucleotide is identified as a potential therapeutic agent
for decreasing the activity of secretin receptor-like GPCR.
13. A method of reducing the activity of secretin receptor-like GPCR, comprising
25 the steps of:
contacting a cell with a reagent which specifically binds to any
polynucleotide of claim 1 or any secretin receptor-like GPCR polypeptide of

14. A reagent that modulates the activity of a secretin receptor-like GPCR polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.
- 5 15. A pharmaceutical composition, comprising:
the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.
- 10 16. Use of the expression vector of claim 2 or the reagent of claim 14 for the preparation of a medicament for modulating the activity of a secretin receptor-like GPCR in a disease.
- 15 17. Use of claim 16 wherein the disease is urinary incontinence, benign prostate hyperplasia, obesity, cancer, diabetes, osteoporosis, anxiety, depression, hypertension, migraine, compulsive disorder, schizophrenia, autism, a neurodegenerative disorder, or cancer chemotherapy-induced vomiting.
- 20 18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10.
19. The cDNA of claim 18 which comprises SEQ ID NO:1 or 9.
20. The cDNA of claim 18 which consists of SEQ ID NO:1 or 9.
- 25 21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10.
- 30 22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1 or 9.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10.
- 5 24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1 or 9.
25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10.
- 10 26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2 or 10.
27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2 or 10.
- 15 28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10, comprising the steps of:
culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and
20 isolating the polypeptide.
29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1 or 9.
- 25 30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10, comprising the steps of:
hybridizing a polynucleotide comprising 10 contiguous nucleotides of SEQ
30 detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.
- 5 32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10, comprising:
a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 or 9;
and
instructions for the method of claim 30.
- 10 33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10, comprising the steps of:
contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and
detecting the reagent-polypeptide complex.
- 15 34. The method of claim 33 wherein the reagent is an antibody.
35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10, comprising:
20 an antibody which specifically binds to the polypeptide; and
instructions for the method of claim 33.
- 25 36. A method of screening for agents which can modulate the activity of a human secretin receptor-like GPCR, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 40% identical to the amino acid sequence shown in SEQ ID NO:2 or 10 and (2) the amino acid sequence shown in SEQ ID NO:2 or 10; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human secretin receptor-like GPCR.

- 5 37. The method of claim 36 wherein the step of contacting is in a cell.
38. The method of claim 36 wherein the cell is *in vitro*.
39. The method of claim 36 wherein the step of contacting is in a cell-free
10 system.
40. The method of claim 36 wherein the polypeptide comprises a detectable label.
41. The method of claim 36 wherein the test compound comprises a detectable
15 label.
42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.
43. The method of claim 36 wherein the polypeptide is bound to a solid support.
20
44. The method of claim 36 wherein the test compound is bound to a solid support.
45. A method of screening for agents which modulate an activity of a human
secretin receptor-like GPCR, comprising the steps of:
contacting a test compound with a polypeptide comprising an amino acid
sequence consisting of at least one or two peptides selected from SEQ ID NO:1-
which are at least about 40% identical to the amino acid sequence shown in
SEQ ID NO:2 or 10 and (2) the amino acid sequence shown in SEQ ID NO:2
or 10; and

5 detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human secretin receptor-like GPCR, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human secretin receptor-like GPCR.

46. The method of claim 45 wherein the step of contacting is in a cell.

10 47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

15 49. A method of screening for agents which modulate an activity of a human secretin receptor-like GPCR, comprising the steps of:
contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1 or 9; and
detecting binding of the test compound to the product, wherein a test
20 compound which binds to the product is identified as a potential agent for regulating the activity of the human secretin receptor-like GPCR.

50. The method of claim 49 wherein the product is a polypeptide.

25 51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human secretin receptor-like GPCR, comprising the step of:
contacting a cell with a reagent which specifically binds to a product encoded
30 by a polynucleotide comprising the nucleotide sequence shown in SEQ ID

NO:1 or 9, whereby the activity of a human secretin receptor-like GPCR is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

5

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

10 56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

15

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:

20

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10; and
a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

25

62. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide
a pharmaceutically acceptable carrier.

30

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.
- 5 64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.
65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.
- 10 66. A pharmaceutical composition, comprising:
an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 or 10; and
a pharmaceutically acceptable carrier.
- 15 67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO:1 or 9.
- 20 68. A method of treating a secretin receptor-like GPCR dysfunction related disease, wherein the disease is selected from urinary incontinence, benign prostate hyperplasia, obesity, cancer, diabetes, osteoporosis, anxiety, depression, hypertension, migraine, compulsive disorder, schizophrenia, autism, a neurodegenerative disorder, or cancer chemotherapy-induced vomiting comprising the step of:
administering to a patient in need thereof a therapeutically effective dose of a
25 reagent that modulates a function of a human secretin receptor-like GPCR, whereby symptoms of the secretin receptor-like GPCR dysfunction related disease are ameliorated.
- 30 69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

- 98 -

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

5

Fig. 1

atgcctggaacaagtctggattctccacataggataggatctagcact
ggctgtccactctttaccagaaactgggtatgtccctcacctggaatgt
tcccactatagatccaagattcacctaaaagctggagataaacttcaa
agccctgaagggaaacccaagactggaaggatccaagagaaatgcgaa
ggaccttgtattttcttctccaactgcagccagccctgtgctaaggac
tttcatggagaaataggattttacatgtaatcaaaaaaagtggcaaaaa
tcagctgaaacatgtacaagcctttctgtggaaaaactctttaaggac
tcaactgggtgcatctcgccctttctgtagcagcaccatctatacctctg
catattctagacttttcgagctccagagaccattgagagtgtagctcaa
ggaatccgtaagaactgcccctttgattatgcctgcatcactgacatg
gtgaaatcatcagaaacaacatctggaaatattgcattttatagtggag
ttattaaaaaatattttctacagacttgtctgataatgttactcgagag
aaaatgaagagctatagtgaagtggccaaccacatcctcgacacagca
gccattttcaaactgggctttcattcccaacaaaaatgccagctcggat
ttgttgcagtcagtgaaattgtttgccagacaactccacatccacaat
aattctgagaacattgtgaatgaactcttcattcagacaaaagggttt
cacatcaaccataatacctcagagaaaagcctcaattttctcatgagc
atgaacaataaccacagaagatatcttaggaatggtacagattcccagg
caagagctaagggaagctgtggccaaatgcatccaagccattagcata
gctttcccaaccttgggggctatcctgagagaagcccacttgcaaaat
gtgagtccttcccagacaggtaaatggtctggtgctatcagtggtttta
ccagaaagggttgcaagaaatcatactcaccttcgaaaagatcaataaa
acccgcaatgccagagcccagtggtgttggctggcactccaagaaaagg
agatgggatgagaaagcgtgccaaatgatgttggatatcaggaacgaa
gtgaaatgccgctgtaactacaccagtggtggtgatgtctttttccatt
ctcatgtcctccaaatcgatgaccgacaaagtcttggtactacatcacc
tgcatgtgggctcagcgtctcaatcctaagcttggttctttgcctgatc
attgaagccacagtggtgttccgggtggttgtgacggagatatcatac
atgcgtcacgtgtgcatcgtgaatatagcagtggtcccttctgactgcc
aatgtgtggtttatcataggctctcactttaacattaaggcccaggac
tacaacatgtgtgttgagtgacatttttcagccactttttctacctc
tctctgtttttctggatgctcttcaaagcattgctcatcatttatgga
atattggtcattttccgtaggatgatgaagtcccgaatgatggtcatt
ggctttgccattggctatgggtgccattgatcattgctgtcactaca
gttgctatcacagagccagagaaaggctacatgagacctgaggcctgt
tggcttaactgggacaataaccaaagcccttttagcattttgccatcccg
gcgttcgtcattgtggctgtaaatctgattgtggttttgggtgttgc
gtcaacactcagaggccctctattggcagttccaagtctcaggatgtg
gtcataaattatgaggatcagcaaaaatggtgccatcctcactccactg
ctgggactgacctgggggttttggaaatagccactctcatagaaggcact
tccttgacgttccatataatttttgccttgctcaatgctttccagata
agagatgctttgaggatgaggatgtcttcactgaaggggaaatcgagg
gcagctgagccctggagagtccgtgtccttgccggttatgcagagaca
aataccatagctcagtactcaggatctgtgggcaaaggagatcaataa

Fig. 2

MPGTSLDSPHRIGSSTGCPLFTRNWWYVPHLECSHYRSKIHLLKAGDKLQ
SPEGKPKTGRIQEKCEGPCISSSNCSQPCAKDFHGEIGFTCNQKKWQK
SAETCTSLSVKLFKDSTGASRLSVAAPSIPLHILDFRAPETIESVAQ
GIRKNCPFDYACITDMVKSSSETTSGNIAFIVELLKNISTDLSDNVTRE
KMKSYSSEVANHILDTAAISNWAFIPKNASSDLLQSVNLFARQLHIHN
NSENIVNELFIQTKGFHINHNTSEKSLNFSMSMNNTTEDILGMVQIPR
QELRKLWPNASQAISIAFPTLGAILREAHLQNVSLPRQVNGLVLSVVL
PERLQEIILTFEKINKTRNARAQCVGWHSSKKRRWDEKACQMMLDIRNE
VKCRCNYTSVVMSFSILMSSKSMTDKVLDYITCIGLSVVSILSLVLCI
IEATVWSRVVVTEISYMRHVCIVNIAVSLLTANVWFIIGSHFNIKAQD
YNMCVAVTFFSHFFYLSLFFWMLFKALLIYGILVIFRRMMKSRMMVI
GFAIGYGCPLIIAVTTVAITEPEKGYMRPEACWLNWDNTKALLAFAIP
AFVIVAVNLIVVLVVAVNTQRPSIGSSKSQDVVIIMRISKNVAILTPL
LGLTWGFGIATLIEGTSLTFHIIIFALLNAFQIRDALRMRMSSLKGKSR
AAEPWRVRVLAGYAETNTIAQYSGSVGKEDQ

Fig. 3

MRPHLSPPLOQLLLPVLLACAAHSTGALPRLCDVLQVLWEEQDQCLQE
LSREQTGDLGTEQPVPGCEGMWDNISWPSSVPGRMVEVECPFLRML
TSRNGSLFRNCTQDGWSETFPRPNLACGVNVNDSSNEKRHSYLLKLKV
MYTVGYSSSLVMLLVALGILCAFRRLHCTRNYIHMHLFVSFILRALSN
FIKDAVLFSSDDVTYCDAGRAGCKLVMVLFQYCMANYSWLLVEGLYL
HTLLAISFFSERKYLQGFVAFGWGSPAIFVALWAIARHFLEDVGCWDI
NANASIWWIIRGPVILSILINFILFINILRIILMRKLRTQETRGNEVSH
YKRLARSTLLLIPLFGIHYIVFAFSPEDAMEIQLEFFELALGSFQGLVV
AVLYCFLNGEVQLEVQKKWQQWHLREFPLHPVASFSNSTKASHLEQSQ
GTCRTSII

Fig. 5

GTGGCTGTAAATCTGATTGTGGTTTTGGTTGTTGCTGTCAACACTCAG
AGGCCCTCTATTGGCAGTTCCAAGTCTCAGGATGTGGTCATAATTATG
AGGATCAGCAAAAATGTTGCCATCCTCCTCCACTGCTGGGACTGACC
TGGGGTTTTGGAATAGCCACTCTCATAGAAGGCACTTCCTTGACGTTT
CATATAATTTTTGCCTTGCTCAATGCTTTCAGGGTTTTTTCATCCTG
CTGTTTGAACCATTTATGGATCACAAGATAAGAGATGCTTTGAGGATG
AGGATGTCTTCACTGAAGGGGAAATCGAGGGCAGCTGAGAATGCATCA
CTAGGCCCCAACCAATGGATCTAAATTAATGAATCCGTCAAGGATGAAA
TGCTGCCCCATTTCTCATGGATGTCCTGAGACCAAGAGGGGAGATCCA
GGAAGAAAGANGCCATGGAAAGCAGGCTGGAGTGAAGGAAGGAATGGT
CATGCTTCCNTGGGAAGACTTTCCTCCTTGTCAGGAGTGACTCCCA
AGCTCTTGGGTCNGCCGAAGAAACTGAGGATACATTTGCTGACTGGG
C

Fig. 6

ANTTCGGCACAGGNGTGGCTGTAAATCTGATTGTGGTTTTGGTTGTTG
CTGTCAACACTCAGAGGCCCTCTATTGGCAGTTCCAAGTCTCAGGATG
TGGTCATAATTATGAGGATCAGCAAAAATGTTGCCATCCTCACTCCAC
TGCTGGGACTGACCTGGGGTTTTGGAATAGCCACTCTCATAGAAGGCA
CTTCCTTGACGTTCCATATAATTTTTGCCTTGCTCAATGCTTTCCAGG
GTTTTTTCATCCTGCTGTTTGGGAACCATTATGGATCACAAAGATAAGAG
ATGCTTTGAGGATGAGGATGTCTTCACTGAAGGGGAAATCGAGGGGCAG
CTGAGAATGCATCACTAGGCCCAACCAATGGATCTAAATTAATGAATC
GTCAAGGATGAAATGCTGCCCCATTTCTCATGGATGTCCTGAGACCAA
GAGGGGN

Fig. 7

GTGGCTGTAAATCTGATTGTGGTTTTGGTTGTTGCTGTCAACACTCAG
AGGCCCTCTATTGGCAGTTCCAAGTCTCAGGATGTGGTCATAATTATG
AGGATCAGCAAAAATGTTGCCATCCTCACTCCACTGCTGGGACTGACC
TGGGGTTTTGGAATAGCCACTCTCATAGAAGGCACTTCCTTGACGTTT
CATATAATTTTTGCCTTGCTCAATGCTTTCCAGGGTTTTTTCATCCTG
CTGTTTGAACCATTTATGGATCACAAGATAAGAGATGCTTTGAGGATG
AGGATGTCTTCACTGAAGGGGAAATCGAGGGCAGCTGAGAATGCATCA
CTAGGCCCAACCAATGGATCTAAATTAATGAATCGTCAAGGATGAAAT
GCTGCCCCATTTCTCATGGATGTCCTNAGACCAAGAGGGGAGATNCAG
GAGAAAGAGGCC

Fig. 8

GTGGCTGTAAATCTGATTGTGGTTTTGGTTGTTGCTGTCAACACTCAG
AGGCCCTCTATTGGCAGTTCCAAGTCTCAGGATGTGGTCATAATTATG
AGGATCAGCAAAAATGTTGCCATCCTCACTCCACTGCTGGGACTGACC
TGGGGTTTTGGAATAGCCACTCTCATAGAAGGCACTTCCTTGACG TTC
CATATAATTTTTGCCTTGCTCAATGCTTCCAGGGTTTTTTCATNCCT
GCTGTTTGGAAACAC

Fig. 9

AGGATTCGGCTCGTCGGACACACTGTGGGCTTCTATGATCAGGCAAAG
AACCAAGCTTAGGATTGAGACGCTGAGCCCAATGCAGGTGATGTAGTC
CAGAACTTTGTCGGTCATCGATTTGGAGGACATGAGAATGGAAAAAGA
CATCACCACACTGGTGTAGTTACAGCGGCATTTCACTTCG TTCCTGAT
GGTCCGAGCGGA

Fig. 10

atgcctggaacaagtctggattctccacataggataggatctagcact
ggctgtccactctttaccagaaactgggtatgtccctcacctggaatgt
tcccactatagatccaagattcacctaaaagctggagataaacttcaa
agccctgaagggaacccaagactggaaggatccaagagaaatgcgaa
ggacctgtattttcttcttccaactgcagccagccctgtgctaaggac
tttcatggagaaataggatttacatgtaataaaaaaagtggcaaaaa
tcagctgaaacatgtacaagcctttctgtggaaaaactctttaaggac
tcaactgggtgcatctcgcctttctgtagcagcaccatctatacctctg
catattctagactttcgcagctccagagaccattgagagtgtagctcaa
ggaatccgtaagaactgccccctttgattatgcctgcatcactgacatg
gtgaaatcatcagaaacaacatctggaaatattgcattttatagtggag
ttattaaaaaatattttctacagacttgtctgataatgttactcgagag
aaaatgaagagctatagtgaagtggccaaccacatcctcgacacagca
gccatttcaaactgggctttcatttcccaacaaaaatgccagctcggat
ttgtttgcagtcagtgaaatttgtttgccagacaactccacatccacaat
aattctgagaacattgtgaatgaactcttcattcagacaaaagggttt
cacatcaaccataatacctcagagaaaagcctcaatttctccatgagc
atgaacaataccacagaagatatcttaggaatggtagacattcccagg
caagagctaaggaagctgtggccaaatgcatcccaagccatttagcata
gctttcccaaccttgggggctatcctgagagaagcccacttgcaaaat
gtgagtccttccagacaggtaaatgggtctggtgctatcagtggtttta
ccagaaagggttgcaagaaatcatactcaccttcgaaaagatcaataaa
accgcaatgccagagcccagtggtgtgtggctggcactccaagaaaagg
agatgggatgagaaagcgtgccaatgatgttgatatacaggaacgaa
gtgaaatgccgctgtactacaccagtggtgtggtgatgtctttttccatt
ctcatgtcctccaaatcgatgaccgacaaagttctggactacatcacc
tgcatgtgggctcagcgtctcaatcctaagcttggttctttgacctgatc
attgaagccacagtggtgtgtcccgggtgggtgtgacggagatatcatac
atgcgtcacgtgtgcatcgtgaatatagcagtggtcccttctgactgcc
aatgtgtgggtttatcataggctctcactttaacattaaggcccaggac
tacaacatgtgtgttgacgtgacatttttcagccactttttctacctc
tctctgtttttctggatgctcttcaaagcattgctcatcatttatgga
atattgggtcattttccgtaggatgatgaagtcccgaatgatgggtcatt
ggctttgccattggctatgggtgcccatgatcattgctgtcactaca
gttgctatcacagagccagagaaaggctacatgagacctgaggcctgt
tggcttaactgggacaataccaaagcccttttagcatttgccatccc
gcgttcgtcattgtggctgtaaatctgattgtgggttttggtgtgtgct
gtcaacactcagaggccctctatttggcagttccaagtctcaggatgtg
gtcataaattatgaggatcagcaaaaatgttgccatcctcactccactg
ctgggactgacctgggggttttggaatagccactctcatagaaggcact
tccttgacgttccatataatttttgccttgctcaatgctttccagggt
tttttcatcctgctgttttgaaccattatggatcacaagataagagat
gctttgaggatgaggatgtcttactgaaggggaaatcgagggcagct
gagaatgcatcactaggcccaaccaatgga

Fig. 11

MPGTSLDSPH	RIGSSTGCPL	FTRNWYVPHL	ECSHYRSKIH
LKAGDKLQSP	EGKPKTGRIQ	EKCEGPCISS	SNCSQPCAKD
FHGEIGFTCN	QKKWQKSAET	CTSLSVEKLF	KDSTGASRLS
VAAPSIPLHI	LDFRAPETIE	SVAQGIRKNC	PFDYACITDM
VKSSETTSGN	IAFIVELLKN	ISTDLSDNVT	REKMKSyseV
ANHILDTAAI	SNWAFIPNKN	ASSDLLQSVN	LFARQLHIHN
NSENIVNELF	IQTKGFHINH	NTSEKSLNFS	MSMNNTTEDI
LGMVQIPRQE	LRKLWPNASQ	AISIAFPTLG	AILREAHLQN
VSLPRQVNGI	VLSVVLPERL	QEIIILTFEKI	NKTRNARAQC
VGWHSKKRRW	DEKACQMMLD	IRNEVKCRCN	YTSVVMFSFI
LMSSKSMTDK	VLDYITCIGL	SVSILSLVLC	LIIEATVWSR
VVVTEISYMR	HVCIVNIAVS	LLTANVWFII	GSHFNKAQD
YNMCVAVTFF	SHFFYLSLFF	WMLFKALLII	YGILVIFRRM
MKSRMMVIGF	AIGYGCPLII	AVTTVAITEP	EKGYMRPEAC
WLNWDNTKAL	LAFaipAFVI	VAVNLIVVLV	VAVNTQRPSI
GSSKSQDVVI	IMRISKNAVI	LTPLLGLTWG	FGIATLIEGT
SLTFHIIIFAL	LNAFQGFFIL	LFGTIMDHKI	RDALRMRMSS
LKGKSRAAEN	ASLGPTNG		

- 12/21 -

Fig. 12

```

BLASTP          gnment of 193 against trembl|AB019120|AB019120_1
                seven transmembrane receptor"; Rattus norvegicus mRNA for
                smembrane receptor, complete cds. //:gp|AB019120|5525078 product:
                smembrane receptor"; Rattus norvegicus mRNA for seven
                ine receptor, complete cds.

                tit is scoring at : 1e-91 (expectation value)
                ment length (overlap) : 617
                ties : 35 %
                ig matrix : BLOSUM62 (used to infer consensus pattern)
                ise searched : nrdb

:      QKCEGPCISSNCSPCAKDFHGEIGFTCNQKKWKQSAETCTSLSVEKLFKDSTGASR
:      Q: C: . : S: S      . : . : G: . : C      : W: . : . : . : C: S: . : . : L: . : . : . :
:      QKLCQFAGVSRSP-----GQTIGGTVTYKCVGSQWKEETRACISAPINGLLQLAKALIK

      SVAAPSIPHLHILDFRAPETIESVAQGIKKNCPFDYACITDMVKSSSETTSGNIAFIVELL
      . : . : . : P : : D.      SV: G : . : . : . : . : SS: . : G I. I: : LL
      PSQDQKLPKYLRDL-----SVSTGKEEQD-----IRSSPGSLGAIISILDLL

      NISTDLSDNVTREKMKSYSEVANHILDTAAISNWAFI--PNKNASSDLLLQSVNLFARQL
      . : . : T.      V. . E. M: . . . . N ILD: . : . : W. :      . : . N. SS. . LQSV. F: . : . L
      TVPTQ-----VNSEMMRDILATINVILDKSTLNSWEKLLQQQSNQSSQFLQSVVERFSKAL

      IHNENSENIVNELFIQTKGFHINHNTSEKSLNFSMSMNNTTEDILGMVQIPRQELRKLWP
      : : : : . : . : : Q. K. . . I. . . : . : . : . : . : T. . D: G V. I. . : L . L P
      LGDSTPPFLFHPNVQMKSMVIKRCHAQM---YQQKFVFTDSDLWGDVAIDECQLGSLQP

      ASQAISIAFPPTLGAILREAHLQNVSLPRQVNGLVLSVVLPERLQE---IILTFEKINKT
      : S : : : : AFPTL AIL.      Q: . . . . N. LV: . : . : . : . : I : TF K N. .
      SS-IVTVAFPPTLKAILA-----QDQQRKTPSNSLSVMTTTIVSHNIVKPFPRISMFTF-KNNHR

```

Fig. 12 (continued)

```

RNARAQCVGWH-----SKRRWDEKACOMM---LDIRNEVKRCNCTSVVMSFSILMSSKS
...:QCV W: ...:WD...C...:D R:V C:CN...:SFSILMS...S
SGKPPQCVFWNFSLANNTGGWDSSGCTVEDDGRDNRDRVFCCKNHLT---SFSILMSPDS

-----MTDKVLDYITCIGLSVLSLVLCLIEATVWSRVVVTETSYMRHVCIVNIAVSL
...:LD.I: IGL..SI:SL..CL:EA.VW..V...SYMRH:CIVNIA:.L
PDPGSLKILLDDIISYIGLGSIVSLAACLVVEAMVWKSVTKNRTSYMRHICIVNIALCL

LTANVWFIIGS--HFNIKAQDYNMCAVAVTXXSHXXYLXKALLIYIGILVIXRR
L.A:WEI:...H...:..CVA.T..H..YLS:..WML..L:...Y...I...
LIADIWFIWAGAIHDGHYPLNETACVAATFFIHFFYLSVFFWMLTLGLMLFYRLIFILHD

MMKSRMMVIGFAIGYGCPLIIAVTTVAITEPEKGYMRPEACWLNWDNTKALLAFAIPAFAV
..KS....I.F::GYGCPLII::TV::T:P::YMR..ACWLNW::T:ALLAFAIPA::
ASKSTQKAIAFSLGYGCPLIISSITVGVTPQPEVYMRKNACWLNWEDTRALLAFAIPALI

IVAVNLIVVLVAVNTQRPISIGSSK-SQDVVVIIMRISKNVAILTPLLGLTWGFGIATLIE
IV.VN: ...:VV...RPS:G...Q: ...:ISK...:LTPLLGLTWGFG:AT:I:
IVVNVSITVVVITKILRPSVGDKPKQEKSSLFQISKSIGVLTPLLGLTWGFGLATVIQ

GTSLTFHIIIFALLNAFQ      655
G:...FHIIF.LLNAFQ
GSNAVFHIIIFTLLNAFQ      1261

```

- 14/21 -

Fig. 13

HMMPFAM
 7 transmembrane receptor (Secretin family)
 bit is scoring at : 32.5
 ng matrix : BLOSUM62 (used to infer consensus pattern)
 Q: DKVLDYITCIGLSVS-ILSLVLCIIeATVWSrvvvtEISYMRHVCIVNIAVSLLTANVW
 L..I :G.S:S :L:L..I :R:..N: :S:..:
 lllkviytVGyslSsLvcllaiaifllfr....kLrctRnyIHmNflsflrals
 IIGSH-FNIKAqdynmCVAVTFFSHFFYLSLFFWMLFKALLIYGIL---VIFRRMMKS
 :IG. :C.V..F H:F:L: FFWML.:L.:Y :L V :.
 LlgdaviInsg....CkvvavflhyfflaNFFWmLvEG.lYLyTLlvvtvevffserk
 RMMVIGFAIGYGCPLIIaVTTVAITEPEK-----GYMRPEACWLNW-DNTKALLA
 :.. :IG:G.P.: VT: AI..P:K GY .. .CWL: .N:
 lwwY.lliGWGvPavf.vtiwaivrpdkygpilaeapagygnegcCWlsndtnsgfwWi
 AIPAFVIVAVNLIV-VLVVAVNTQRPISGSSKSQDVV---IIMR--ISKNVAILTPLLG
 .P.:I.:VN.I.: :Q: .I .S S... :K:..L.PLLG
 kGPillilvNfffinilriLvqklri.dslspqtgetdgyrkrlvkstLlIlPLLG
 TWGFGIATLIEG----TSLTFHIIIFALLNAFQ-----IRDA 659
 TW : : .SL.F :F:LN:FQ :.
 twilflfapedqsqGtlslvflyfliLnSfQGffvavlyCfIngev 273

- 15/21 -

Fig. 14

HMMPFAM - alignment of 193 against pfam|hmm|GPS
Latrophilin/CL-1-like GPS domain

This hit is scoring at : 4.4

Scoring matrix : BLOSUM62 (used to infer consensus pattern)

Q:	356	ARAQCVGWHKK---RRWDE-KACQMMMLDIR-NEVKRCNYTSvvmSFSILMSSKSM	407
	:	... CV W...: W...:C::: : :...C.CN::: SF::LM. ..	
H:	1	snpiCvfwdeselslgvWstdrGCelvetkspshttcCnHLT...sFAvLmdvspn	54

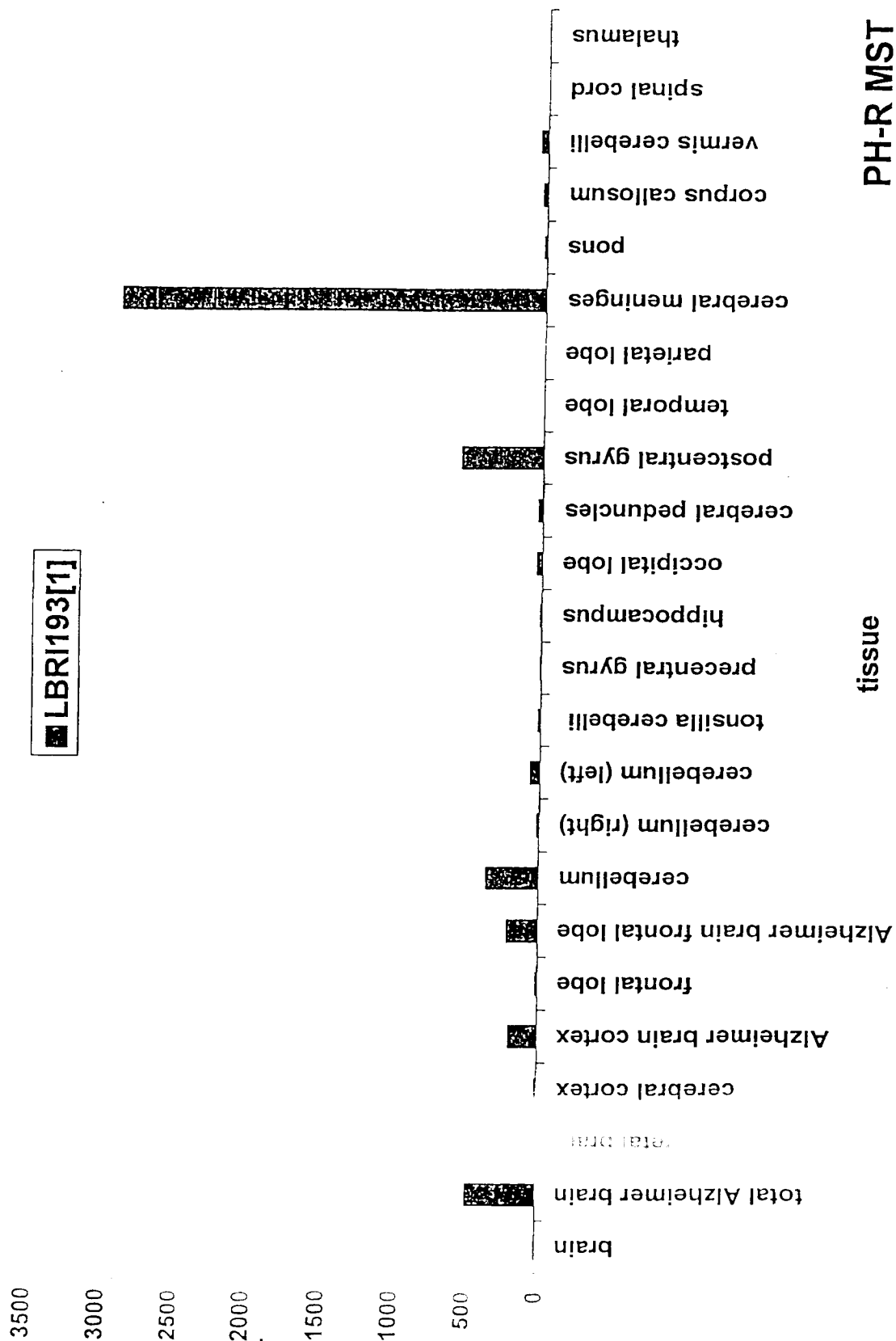
Fig. 15

blocks database			
AC#	Description	Strength	Score RF
BL00649C	0 GPCR family 2 proteins	1370	1214 0
CvAVTFFSHFFYLSlFFWMLfkALLi			483

[illegible]

- 18/21 -

Fig. 17F



- 19/21 -

Fig. 17B



- 20/21 -

Fig. 17C

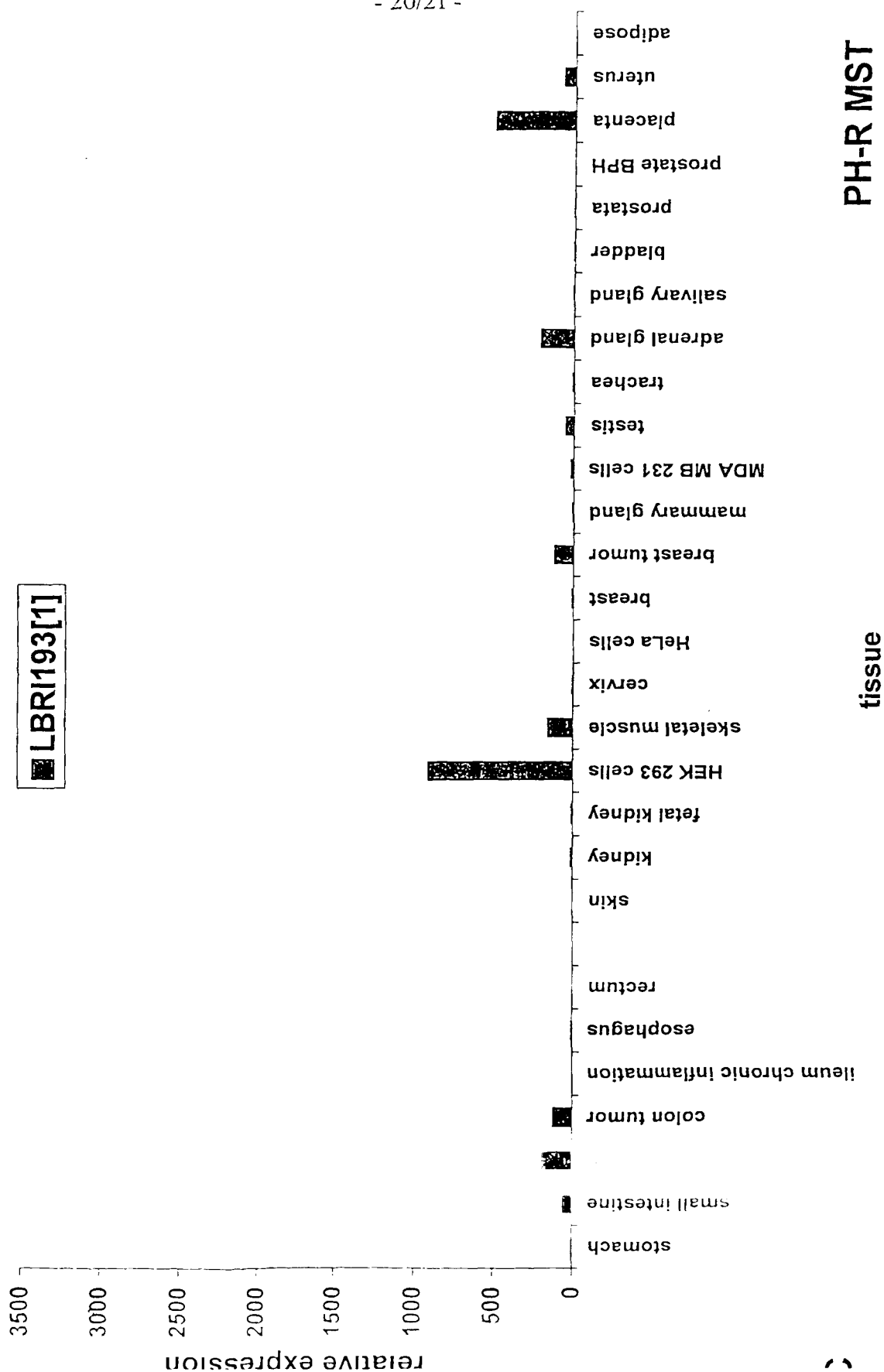
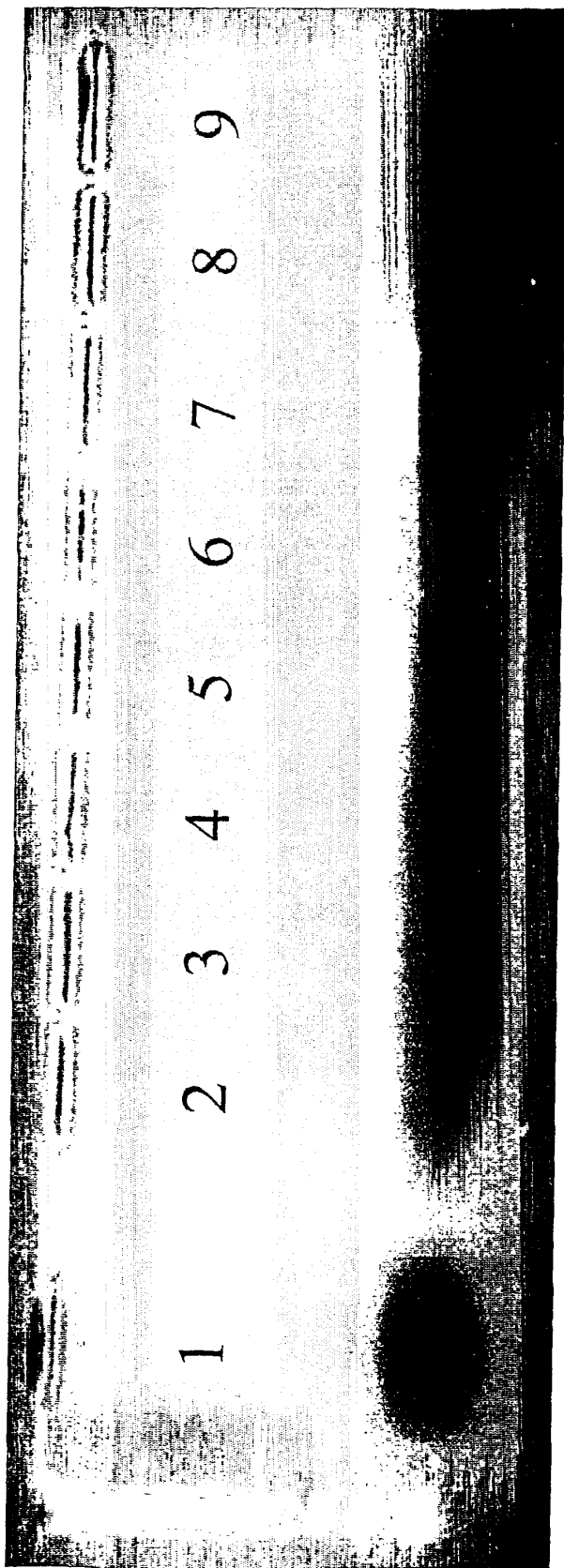


Fig. 18



SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN SECRETIN RECEPTOR-LIKE GPCR

<130> LI0163 Foreign Countries

<150> US 60/238,045

<151> 2000-10-06

<150> US 60/XXX,XXX

<151> 2001-08-31

<160> 10

<170> PatentIn version 3.1

<210> 1

$\langle 211 \rangle$ 2112

<212> DNA

<213> Homo sapiens

<400> 1

atgcctggaa	caagtctgga	ttctccacat	aggataggat	ctagcactgg	ctgtccactc	60
tttaccagaa	actggtatgt	ccctcacctg	gaatgttccc	actatagatc	caagattcac	120
ctaaaagctg	gagataaact	tcaaagccct	gaagggaaac	ccaagactgg	aaggatccaa	180
gagaaatgcg	aaggaccttg	tatttcttct	tccaactgca	gccagccctg	tgctaaggac	240
tttcatggag	aaataggatt	tacatgtaat	caaaaaaagt	ggcaaaaatc	agctgaaaca	300
tgtacaagcc	tttctgtgga	aaaactcttt	aaggactcaa	ctggtgcata	tcgcctttct	360
gtagcagcac	catctatacc	tctgcatatt	ctagactttc	gagctccaga	gaccattgag	420
agtgtagctc	aaggaatccg	taagaactgc	ccctttgatt	atgcctgcat	cactgacatg	480
gtgaaatcat	cagaaacaac	atctggaaat	attgcattta	tagtggagtt	attaaaaaat	540
attttotacag	acttgtctga	taatgttact	cgagagaaaa	tgaagagcta	tagtgaagtg	600
gccaaaccaca	tcctcgacac	agcagccatt	tcaaactggg	ctttcattcc	caacaaaaat	660
gccagctcgg	atttgttgca	gtcagtgaat	ttgtttgcc	gacaactcca	catccacaat	720
aattctgaga	acattgtgaa	tgaactcttc	attcagacaa	aagggtttca	catcaaccat	780
aatacctcag	agaaaagcct	caatttctcc	atgagcatga	acaataccac	agaagatatc	840
ttaggaatgg	tacagattcc	caggcaagag	ctaaggaagc	tgtggccaaa	tgcatcccaa	900

caagaaatca tactcacctt cgaaaagatc aataaaaacc gcaatgccag agcccagtgt 1080
gttggtgtggc actccaagaa aaggagatgg gatagagaaag cgtgccaaat gatgttggaat 1140

1. *Introduction* 1

```

ctcatgtcct ccaaatcgat gaccgacaaa gttctggact acatcacctg cattgggctc 1260
agcgtctcaa tcctaagctt ggttctttgc ctgatcattg aagccacagt gtgggtcccg 1320
gtgggtgtga cggagatatc atacatgcgt cacgtgtgca tcgtgaatat agcagtgtcc 1380
cttctgactg ccaatgtgtg gtttatcata ggctctcaact ttaacattaa ggcccaggac 1440
tacaacatgt gtgttgcaat gacatttttc agccactttt tctacctctc tctgtttttc 1500
tggatgctct tcaaagcatt gctcatcatt tatggaatat tggtcatttt ccgtaggatg 1560
atgaagtccc gaatgatggt cattggcttt gccattggt atgggtgccc attgatcatt 1620
gctgtcacta cagttgctat cacagagcca gagaaaggct acatgagacc tgaggcctgt 1680
tggcttaact gggacaatac caaagccott ttagcatttg ccatcccggc gttcgtcatt 1740
gtggctgtaa atctgattgt ggttttggtt gttgctgtca aactcagag gccctctatt 1800
ggcagttcca agtctcagga tgtggtcata attatgagga tcagcaaaaa tgttgccatc 1860
ctcactccac tgctgggact gacctggggt tttggaatag ccactctcat agaaggcact 1920
tccttgacgt tccatataat ttttgccctg ctcaatgctt tccagataag agatgctttg 1980
aggatgagga tgtcttcact gaaggggaaa tcgagggcag ctgagccctg gagagtccgt 2040
gtccttgccg gttatgcaga gacaaatacc atagctcagt actcaggatc tgtgggcaaa 2100
gaggatcaat aa 2112

```

<210> 2
 <211> 703
 <212> PRT
 <213> Homo sapiens

<400> 2

```

Met Pro Gly Thr Ser Leu Asp Ser Pro His Arg Ile Gly Ser Ser Thr
1           5           10           15

```

```

Gly Cys Pro Leu Phe Thr Arg Asn Trp Tyr Val Pro His Leu Glu Cys
20           25           30

```

```

Ser His Tyr Arg Ser Lys Ile His Leu Lys Ala Gly Asp Lys Leu Gln
35           40           45

```

```

Ser Pro Glu Gly Lys Pro Lys Thr Gly Arg Ile Gln Glu Lys Cys Glu
50           55           60

```

```

Gly Pro Cys Ile Ser Ser Ser Asn Cys Ser Gln Pro Cys Ala Lys Asp
65           70           75           80

```

```

Phe His Gly Glu Ile Gly Phe Thr Cys Asn Gln Lys Lys Trp Gln Lys
85           90           95

```

Ser Ala Glu Thr Cys Thr Ser Leu Ser Val Glu Lys Leu Phe Lys Asp
100 105 110

Ser Thr Gly Ala Ser Arg Leu Ser Val Ala Ala Pro Ser Ile Pro Leu
115 120 125

His Ile Leu Asp Phe Arg Ala Pro Glu Thr Ile Glu Ser Val Ala Gln
130 135 140

Gly Ile Arg Lys Asn Cys Pro Phe Asp Tyr Ala Cys Ile Thr Asp Met
145 150 155 160

Val Lys Ser Ser Glu Thr Thr Ser Gly Asn Ile Ala Phe Ile Val Glu
165 170 175

Leu Leu Lys Asn Ile Ser Thr Asp Leu Ser Asp Asn Val Thr Arg Glu
180 185 190

Lys Met Lys Ser Tyr Ser Glu Val Ala Asn His Ile Leu Asp Thr Ala
195 200 205

Ala Ile Ser Asn Trp Ala Phe Ile Pro Asn Lys Asn Ala Ser Ser Asp
210 215 220

Leu Leu Gln Ser Val Asn Leu Phe Ala Arg Gln Leu His Ile His Asn
225 230 235 240

Asn Ser Glu Asn Ile Val Asn Glu Leu Phe Ile Gln Thr Lys Gly Phe
245 250 255

His Ile Asn His Asn Thr Ser Glu Lys Ser Leu Asn Phe Ser Met Ser
260 265 270

Met Asn Asn Thr Thr Glu Asp Ile Leu Gly Met Val Gln Ile Pro Arg
275 280 285

Gln Glu Leu Arg Lys Leu Trp Pro Asn Ala Ser Gln Ala Ile Ser Ile
290 295 300

Val Ser Leu Pro Arg Gln Val Asn Gly Leu Val Leu Ser Val Val Leu
325 330 335

340

345

350

Thr Arg Asn Ala Arg Ala Gln Cys Val Gly Trp His Ser Lys Lys Arg
 355 360 365

Arg Trp Asp Glu Lys Ala Cys Gln Met Met Leu Asp Ile Arg Asn Glu
 370 375 380

Val Lys Cys Arg Cys Asn Tyr Thr Ser Val Val Met Ser Phe Ser Ile
 385 390 395 400

Leu Met Ser Ser Lys Ser Met Thr Asp Lys Val Leu Asp Tyr Ile Thr
 405 410 415

Cys Ile Gly Leu Ser Val Ser Ile Leu Ser Leu Val Leu Cys Leu Ile
 420 425 430

Ile Glu Ala Thr Val Trp Ser Arg Val Val Val Thr Glu Ile Ser Tyr
 435 440 445

Met Arg His Val Cys Ile Val Asn Ile Ala Val Ser Leu Leu Thr Ala
 450 455 460

Asn Val Trp Phe Ile Ile Gly Ser His Phe Asn Ile Lys Ala Gln Asp
 465 470 475 480

Tyr Asn Met Cys Val Ala Val Thr Phe Phe Ser His Phe Phe Tyr Leu
 485 490 495

Ser Leu Phe Phe Trp Met Leu Phe Lys Ala Leu Leu Ile Ile Tyr Gly
 500 505 510

Ile Leu Val Ile Phe Arg Arg Met Met Lys Ser Arg Met Met Val Ile
 515 520 525

Gly Phe Ala Ile Gly Tyr Gly Cys Pro Leu Ile Ile Ala Val Thr Thr
 530 535 540

Val Ala Ile Thr Glu Pro Glu Lys Gly Tyr Met Arg Pro Glu Ala Cys
 545 550 555 560

Trp Leu Asn Trp Asp Asn Thr Lys Ala Leu Leu Ala Phe Ala Ile Pro
 565 570 575

Ala Phe Val Ile Val Ala Val Asn Leu Ile Val Val Leu Val Val Ala
 580 585 590

Val Asn Thr Gln Arg Pro Ser Ile Gly Ser Ser Lys Ser Gln Asp Val
595 600 605

Val Ile Ile Met Arg Ile Ser Lys Asn Val Ala Ile Leu Thr Pro Leu
610 615 620

Leu Gly Leu Thr Trp Gly Phe Gly Ile Ala Thr Leu Ile Glu Gly Thr
625 630 635 640

Ser Leu Thr Phe His Ile Ile Phe Ala Leu Leu Asn Ala Phe Gln Ile
645 650 655

Arg Asp Ala Leu Arg Met Arg Met Ser Ser Leu Lys Gly Lys Ser Arg
660 665 670

Ala Ala Glu Pro Trp Arg Val Arg Val Leu Ala Gly Tyr Ala Glu Thr
675 680 685

Asn Thr Ile Ala Gln Tyr Ser Gly Ser Val Gly Lys Glu Asp Gln
690 695 700

<210> 3
<211> 440
<212> PRT
<213> Homo sapiens

<400> 3

Met Arg Pro His Leu Ser Pro Pro Leu Gln Gln Leu Leu Leu Pro Val
1 5 10 15

Leu Leu Ala Cys Ala Ala His Ser Thr Gly Ala Leu Pro Arg Leu Cys
20 25 30

Asp Val Leu Gln Val Leu Trp Glu Glu Gln Asp Gln Cys Leu Gln Glu
35 40 45

Leu Ser Arg Glu Gln Thr Gly Asp Leu Gly Thr Glu Gln Pro Val Pro
50 55 60

Gly Cys Glu Gly Met Trp Asp Asn Ile Ser Cys Trp Pro Ser Ser Val
65 70 75 80

Thr Ser Arg Asn Gly Ser Leu Phe Arg Asn Cys Thr Gln Asp Gly Trp
100 105 110

Ser Glu Thr Phe Pro Arg Pro Asn Leu Ala Cys Gly Val Asn Val Asn
 115 120 125

Asp Ser Ser Asn Glu Lys Arg His Ser Tyr Leu Leu Lys Leu Lys Val
 130 135 140

Met Tyr Thr Val Gly Tyr Ser Ser Ser Leu Val Met Leu Leu Val Ala
 145 150 155 160

Leu Gly Ile Leu Cys Ala Phe Arg Arg Leu His Cys Thr Arg Asn Tyr
 165 170 175

Ile His Met His Leu Phe Val Ser Phe Ile Leu Arg Ala Leu Ser Asn
 180 185 190

Phe Ile Lys Asp Ala Val Leu Phe Ser Ser Asp Asp Val Thr Tyr Cys
 195 200 205

Asp Ala His Arg Ala Gly Cys Lys Leu Val Met Val Leu Phe Gln Tyr
 210 215 220

Cys Ile Met Ala Asn Tyr Ser Trp Leu Leu Val Glu Gly Leu Tyr Leu
 225 230 235 240

His Thr Leu Leu Ala Ile Ser Phe Phe Ser Glu Arg Lys Tyr Leu Gln
 245 250 255

Gly Phe Val Ala Phe Gly Trp Gly Ser Pro Ala Ile Phe Val Ala Leu
 260 265 270

Trp Ala Ile Ala Arg His Phe Leu Glu Asp Val Gly Cys Trp Asp Ile
 275 280 285

Asn Ala Asn Ala Ser Ile Trp Trp Ile Ile Arg Gly Pro Val Ile Leu
 290 295 300

Ser Ile Leu Ile Asn Phe Ile Leu Phe Ile Asn Ile Leu Arg Ile Leu
 305 310 315 320

Met Arg Lys Leu Arg Thr Gln Glu Thr Arg Gly Asn Glu Val Ser His
 325 330 335

Tyr Lys Arg Leu Ala Arg Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly
 340 345 350

Ile His Tyr Ile Val Phe Ala Phe Ser Pro Glu Asp Ala Met Glu Ile
 355 360 365

Gln Leu Phe Phe Glu Leu Ala Leu Gly Ser Phe Gln Gly Leu Val Val
 370 375 380

Ala Val Leu Tyr Cys Phe Leu Asn Gly Glu Val Gln Leu Glu Val Gln
 385 390 395 400

Lys Lys Trp Gln Gln Trp His Leu Arg Glu Phe Pro Leu His Pro Val
 405 410 415

Ala Ser Phe Ser Asn Ser Thr Lys Ala Ser His Leu Glu Gln Ser Gln
 420 425 430

Gly Thr Cys Arg Thr Ser Ile Ile
 435 440

<210> 4
 <211> 577
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (443)..(443)
 <223> n=a, c, g or t

<220>
 <221> misc_feature
 <222> (490)..(490)
 <223> n=a, c, g or t

<220>
 <221> misc_feature
 <222> (541)..(541)
 <223> n=a, c, g or t

<400> 4
 gtggctgtaa atctgattgt ggttttgggt gttgctgtca acactcagag gccctctatt 60
 ggcagttcca agtctcagga tgtggtcata attatgagga tcagcaaaaa tgttgccatc 120
 ctcaactccac tgetgggact gacctggggt tttggaatag ccactctcat agaaggcact 180
 tocttgacgt tccatataat ttttgccttg ctcaatgctt tccagggttt tttcatcctg 240
 ctgttttgga ccattatgga tcacaagata agagatgctt tqagqatgag gatgtcttca 300

taatatgaata atcaaaagat caaatctgga caaatctgga caaatctgga caaatctgga 360
 aggggagatc caggaagaaa gangccatgg aaagcaggct ggagtgaagg aaggaatggt 480
 catgcttctg tgggagatc ttatctgga ttatctgga ttatctgga ttatctgga 540

ngccgaagaa aactgaggat acatttgctg actgggc

577

<210> 5
 <211> 439
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (2)..(2)
 <223> n=a, c, g or t

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> n=a, c, g or t

<220>
 <221> misc_feature
 <222> (439)..(439)
 <223> n=a, c, g or t

<400> 5
 anttoggcac agngtggct gtaaactctga ttgtggtttt ggttggtgct gtcaacactc 60
 agaggccctc tattggcagt tccaagtctc aggatgtggt cataattatg aggatcagca 120
 aaaatgttgc catcctcact coactgctgg gactgacctg gggtttttga atagccactc 180
 tcatagaagg cacttccctg acgttccata taatttttgc cttgctcaat gctttccagg 240
 gttttttcat cctgctgttt ggaaccatta tggatcacia gataagagat gctttgagga 300
 tgaggatgtc ttactgaag gggaaatcga gggcagctga gaatgcatca ctaggcccaa 360
 ccaatggatc taaattaatg aatcgtcaag gatgaaatgc tgccccattt ctcatggatg 420
 tcctgagacc aagaggggn 439

<210> 6
 <211> 444
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <222> (411)..(411)
 <223> n=a, c, g or t

<220>
 <221> misc_feature
 <222> (429)..(429)
 <223> n=a, c, g or t

<400> 6
 gtggctgtaa atctgattgt ggttttggtt gttgctgtca aactcagag gccctctatt 60

```

ggcagttcca agtctcagga tgtggtcata attatgagga tcagcaaaaa tgttgccatc      120
ctcactccac tgctgggact gacctgggggt tttggaatag ccactctcat agaaggcact      180
tccttgacgt tccatataat ttttgcttg ctcaatgctt tccagggttt tttcctctg      240
ctgttttgaa ccattatgga tcacaagata agagatgctt tgaggatgag gatgtcttca      300
ctgaagggga aatcgagggc agctgagaat gcatcactag gcccaaccaa tggatctaaa      360
ttaatgaatc gtcaaggatg aaatgctgcc ccatttctca tggatgtcct nagaccaaga      420
ggggagatnc aggagaaaga ggcc                                             444

```

```

<210> 7
<211> 255
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (237)..(237)
<223> n=a, c, g or t

```

```

<400> 7
gtggctgtaa atctgattgt ggttttgggt gttgctgtca acactcagag gccctctatt      60
ggcagttcca agtctcagga tgtggtcata attatgagga tcagcaaaaa tgttgccatc      120
ctcactccac tgctgggact gacctgggggt tttggaatag ccactctcat agaaggcact      180
tccttgacgt tccatataat ttttgcttg ctcaatgctt tccagggttt tttcctctg      240
gtgttttgga aacac                                                         255

```

```

<210> 8
<211> 204
<212> DNA
<213> Homo sapiens

```

```

<400> 8
aggattoggc tcgtcggaca cactgtgggc ttctatgac aggcaaagaa ccaagcttag      60
gattgagacg ctgagcccaa tgcaggatgat gtagtcaga actttgtcgg tcctcgattt      120
ggaggacatg agaatggaaa aagacatcac cacactgggt tagttacagc ggcatttcac      180
ttcgttcttg atggtcogag cgga                                             204

```

```

<210> 9

```

```

<213> Homo sapiens

```

```

<400> 9
atgcctggaa caagtctgga ttctccacat aggataggat ctagcactgg ctgtccactc      60

```

```

<210> 10

```

ctaaaagctg gagataaact tcaaagccct gaagggaaac ccaagactgg aaggatccaa 180
 gagaaatgcg aaggaccttg tatttcttct tccaactgca gccagccctg tgctaaggac 240
 tttcatggag aaataggatt tacatgtaat caaaaaaagt ggcaaaaatc agctgaaaca 300
 tgtacaagcc tttctgtgga aaaactcttt aaggactcaa ctgggtgcac tcgcctttct 360
 gtagcagcac catctatacc tctgcatatt ctagactttc gagctccaga gaccattgag 420
 agtgtagctc aaggaatccg taagaactgc ccctttgatt atgcctgcat cactgacatg 480
 gtgaaatcat cagaaacaac atctggaaat attgcattta tagtggagtt attaaaaaat 540
 atttctacag acttgtctga taatgttact cgagagaaaa tgaagagcta tagtgaagtg 600
 gccaacca ca tctcgacac agcagccatt tcaaactggg ctttcattcc caacaaaaat 660
 gccagctcgg atttgttgca gtcagtgaat ttgtttgcc gacaactcca catccacaat 720
 aattctgaga acattgtgaa tgaactcttc attcagacaa aagggtttca catcaacat 780
 aatacctcag agaaaagcct caatttctcc atgagcatga acaataccac agaagatato 840
 ttaggaatgg tacagattcc caggcaagag ctaaggaagc tgtggccaaa tgcacccaa 900
 gccattagca tagctttccc aaccttgggg gctatcctga gagaagccca cttgcaaaat 960
 gtgagtcttc ccagacaggt aaatggtctg gtgctatcag tggttttacc agaaaggttg 1020
 caagaaatca tactcacctt cgaaaagatc aataaaaccc gcaatgccag agcccagtgt 1080
 gttggctggc actccaagaa aaggagatgg gatgagaaaag cgtgccaaat gatgttggt 1140
 atcaggaacg aagtgaaatg ccgctgtaac tacaccagtg tggatgatgc tttttccatt 1200
 ctcatgtcct ccaaatcgat gaccgacaaa gttctggact acatcacctg cattgggctc 1260
 agcgtctcaa tctaagctt ggttctttgc ctgatcattg aagccacagt gtggtcccg 1320
 gtggttgtga cggagatata atacatgcgt cacgtgtgca tcgtgaatat agcagtgtcc 1380
 cttctgactg ccaatgtgtg gtttatcata ggctctcact ttaacattaa ggcccaggac 1440
 tacaacatgt gtgttgcatg gacatttttc agccactttt tctacctctc tctgtttttc 1500
 tggatgctct tcaaagcatt gtcacatcatt tatggaatat tggtcatttt ccgtaggatg 1560
 atgaagtccc gaatgatggt cattggcttt gccattggct atgggtgccc attgatcatt 1620
 gctgtcacta cagttgctat cacagagcca gagaaaggct acatgagacc tgaggcctgt 1680
 tggcttaact gggacaatac caaagccctt ttagcatttg ccatccggc gttcgtcatt 1740
 gtggctgtaa atctgattgt ggttttggtt gttgctgtca aactcagag gccctctatt 1800
 ggcagttcca agtctcagga tgtggtcata attatgagga tcagcaaaaa tgttgccatc 1860
 ctactccac tgcctgggact gacctggggt tttggaatag ccactctcat agaaggcact 1920
 tecttgacgt tccatataat ttttgcttg ctcaatgctt tccagggttt tttcatcctg 1980

ctgttttgga ccattatgga tcacaagata agagatgctt tgaggatgag gatgtcttca 2040

ctgaagggga aatcgagggc agctgagaat gcatcactag gcccaaccaa tgga 2094

<210> 10
 <211> 698
 <212> PRT
 <213> Homo sapiens

<400> 10

Met Pro Gly Thr Ser Leu Asp Ser Pro His Arg Ile Gly Ser Ser Thr
 1 5 10 15

Gly Cys Pro Leu Phe Thr Arg Asn Trp Tyr Val Pro His Leu Glu Cys
 20 25 30

Ser His Tyr Arg Ser Lys Ile His Leu Lys Ala Gly Asp Lys Leu Gln
 35 40 45

Ser Pro Glu Gly Lys Pro Lys Thr Gly Arg Ile Gln Glu Lys Cys Glu
 50 55 60

Gly Pro Cys Ile Ser Ser Ser Asn Cys Ser Gln Pro Cys Ala Lys Asp
 65 70 75 80

Phe His Gly Glu Ile Gly Phe Thr Cys Asn Gln Lys Lys Trp Gln Lys
 85 90 95

Ser Ala Glu Thr Cys Thr Ser Leu Ser Val Glu Lys Leu Phe Lys Asp
 100 105 110

Ser Thr Gly Ala Ser Arg Leu Ser Val Ala Ala Pro Ser Ile Pro Leu
 115 120 125

His Ile Leu Asp Phe Arg Ala Pro Glu Thr Ile Glu Ser Val Ala Gln
 130 135 140

Gly Ile Arg Lys Asn Cys Pro Phe Asp Tyr Ala Cys Ile Thr Asp Met
 145 150 155 160

Val Lys Ser Ser Glu Thr Thr Ser Gly Asn Ile Ala Phe Ile Val Glu
 165 170 175

180

185

190

Lys Met Lys Ser Tyr Ser Glu Val Ala Asn His Ile Leu Asp Thr Ala
 195 200 205

Ala Ile Ser Asn Trp Ala Phe Ile Pro Asn Lys Asn Ala Ser Ser Asp
 210 215 220

Leu Leu Gln Ser Val Asn Leu Phe Ala Arg Gln Leu His Ile His Asn
 225 230 235 240

Asn Ser Glu Asn Ile Val Asn Glu Leu Phe Ile Gln Thr Lys Gly Phe
 245 250 255

His Ile Asn His Asn Thr Ser Glu Lys Ser Leu Asn Phe Ser Met Ser
 260 265 270

Met Asn Asn Thr Thr Glu Asp Ile Leu Gly Met Val Gln Ile Pro Arg
 275 280 285

Gln Glu Leu Arg Lys Leu Trp Pro Asn Ala Ser Gln Ala Ile Ser Ile
 290 295 300

Ala Phe Pro Thr Leu Gly Ala Ile Leu Arg Glu Ala His Leu Gln Asn
 305 310 315 320

Val Ser Leu Pro Arg Gln Val Asn Gly Leu Val Leu Ser Val Val Leu
 325 330 335

Pro Glu Arg Leu Gln Glu Ile Ile Leu Thr Phe Glu Lys Ile Asn Lys
 340 345 350

Thr Arg Asn Ala Arg Ala Gln Cys Val Gly Trp His Ser Lys Lys Arg
 355 360 365

Arg Trp Asp Glu Lys Ala Cys Gln Met Met Leu Asp Ile Arg Asn Glu
 370 375 380

Val Lys Cys Arg Cys Asn Tyr Thr Ser Val Val Met Ser Phe Ser Ile
 385 390 395 400

Leu Met Ser Ser Lys Ser Met Thr Asp Lys Val Leu Asp Tyr Ile Thr
 405 410 415

Cys Ile Gly Leu Ser Val Ser Ile Leu Ser Leu Val Leu Cys Leu Ile
 420 425 430

Ile Glu Ala Thr Val Trp Ser Arg Val Val Val Thr Glu Ile Ser Tyr
 435 440 445

Met Arg His Val Cys Ile Val Asn Ile Ala Val Ser Leu Leu Thr Ala
 450 455 460

Asn Val Trp Phe Ile Ile Gly Ser His Phe Asn Ile Lys Ala Gln Asp
 465 470 475 480

Tyr Asn Met Cys Val Ala Val Thr Phe Phe Ser His Phe Phe Tyr Leu
 485 490 495

Ser Leu Phe Phe Trp Met Leu Phe Lys Ala Leu Leu Ile Ile Tyr Gly
 500 505 510

Ile Leu Val Ile Phe Arg Arg Met Met Lys Ser Arg Met Met Val Ile
 515 520 525

Gly Phe Ala Ile Gly Tyr Gly Cys Pro Leu Ile Ile Ala Val Thr Thr
 530 535 540

Val Ala Ile Thr Glu Pro Glu Lys Gly Tyr Met Arg Pro Glu Ala Cys
 545 550 555 560

Trp Leu Asn Trp Asp Asn Thr Lys Ala Leu Leu Ala Phe Ala Ile Pro
 565 570 575

Ala Phe Val Ile Val Ala Val Asn Leu Ile Val Val Leu Val Val Ala
 580 585 590

Val Asn Thr Gln Arg Pro Ser Ile Gly Ser Ser Lys Ser Gln Asp Val
 595 600 605

Val Ile Ile Met Arg Ile Ser Lys Asn Val Ala Ile Leu Thr Pro Leu
 610 615 620

Leu Gly Leu Thr Trp Gly Phe Gly Ile Ala Thr Leu Ile Glu Gly Thr
 625 630 635 640

Ser Leu Thr Phe His Ile Ile Phe Ala Leu Leu Asn Ala Phe Gln Gly
 645 650 655

Phe Phe Ile Leu Leu Phe Gly Thr Ile Met Asp His Lys Ile Arg Asp
 660 665 670

Glu Asn Ala Ser Leu Gly Pro Thr Asn Gly
 690 695

